NEUTRON ACTIVATION ANALYSIS OF CATARACTOUS LENSES OF MICE AND MONGOLIAN GERBILS EXPOSED TO ACUTE DOSES OF X-RAYS, THERMAL AND FAST NEUTRONS

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NEUTRON ACTIVATION ANALYSIS OF CATARACTOUS LENSES OF MICE AND MONGOLIAN GERBILS EXPOSED TO ACUTE DOSES OF X-RAYS, THERMAL AND FAST NEUTRONS

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SUMMARY

It has long been known that the mammalian lens is highly susceptible to cataracts induced by ionizing radiation and that these cataracts are often associated with significant changes in lenticular ion concentrations. In the past no real attempts were made to correlate such ion changes with the clinical development of opacity as observed by slit-lamp biomicroscopy, the most common method of cataract diagnosis. Furthermore, since by nature slit-lamp examination is subjective (dependent on evaluation of lens state by the observer) and non-quantitative, no objective and precise method existed for analyzing cataractous stages of the lens which could then be compared with clinical observation.

The present work is an attempt to correlate the clinical progression of opacities in lenses developing radiation-induced cataracts with changes in lenticular ion concentrations. To accomplish this goal the cataractogenic effects of single, acute exposures of 160 kVp x-rays, thermal neutrons and fast neutrons on lenses of mice and Mongolian gerbils have been studied by means of thermal neutron activation analysis and slit-lamp biomicroscopy.

No radiation cataracts were observed in Mongolian gerbil lenses as late as nine months after an acute exposure of 1,000 R of 160 kVp x-rays. However, nine months post-exposure slight but statistically significant (98% confidence level) increases in sodium and chlorine concentrations in whole gerbil lenses were detected by activation analysis. Gerbil lenses

were found to have a large potassium to sodium ratio relative to other mammals such as rabbits, rats, mice and man.

In mice, ionizing radiation cataracts first appeared three and one-half to four months following an acute exposure of 960 rad of 160 kVp x-rays. The latency period between radiation exposure and the initial appearance of lens changes was found to be proportional to the age of the animals at exposure. Highly significant increases in sodium and chlorine and decreases in potassium were detected in mouse lenses five to nine months after an acute exposure of 360 rad of x-rays but prior to the clinical detection of cataracts by slit-lamp examination. In some cases lenses exposed to ionizing radiation underwent a loss of potassium prior to a net influx of sodium and chlorine. No lenses were observed with normal levels of potassium concurrent with abnormally high levels of sodium and chlorine. Ion concentrations in lenses which had developed clinically detectable, incipient radiation cataracts were not significantly different from those lenses which exhibited only pre-cataractous changes such as edema and swollen nuclei.

In mice, after six months, the potassium to sodium ratio decreased from 3.5 ± 0.21 in control lenses to 0.31 ± 0.21 in lenses that had been exposed to 960 rad of x-rays. The dose-response relationship for the K/Na ratio in whole mouse lenses is suggestive of a sigmoidal curve but more data are needed in the low dose range (less than 200 rad) to establish whether there is indeed a threshold (nonlinear) relationship. At high doses the K/Na ratio in the whole lens approaches the equilibrium value found in the aqueous humor of the eye. This behavior is expected on the basis of existing knowledge concerning eye function.

CHAPTER I

INTRODUCTION

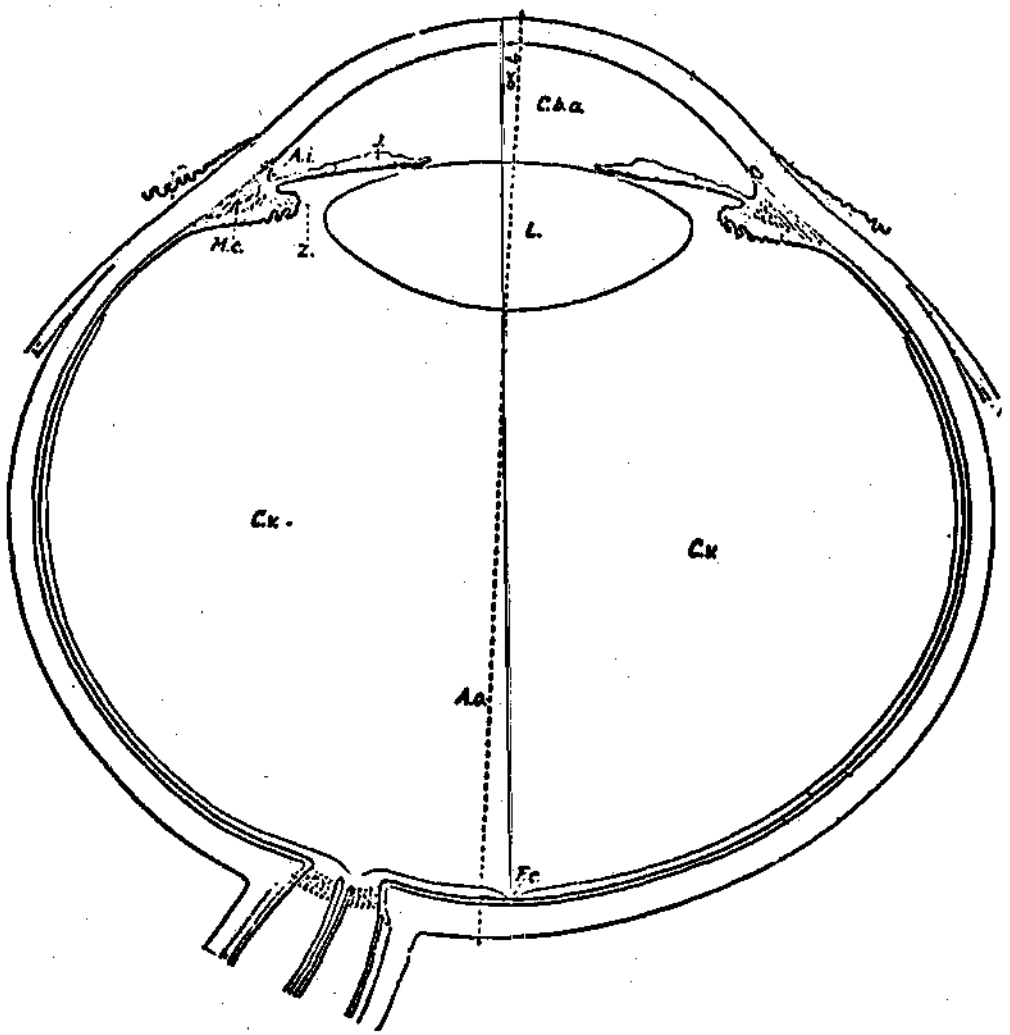
Purpose and Objectives of This Work

There has long been considerable interest in radiation damage effects by ionizing radiations to the eye and, specifically, the induction of cataracts in the lens by both low- and high-LET radiations. This dissertation is concerned with examining the mechanisms of cataract formation in animal lenses following exposure to ionizing radiation, with particular attention to changes in lens composition as reflected by ion concentrations. However, in order to understand the technical terminology and detailed objectives of this work it is necessary to briefly review the main features of lens anatomy and basic physiology.

Anatomy of the Eye and Lens

The basic structure of the eye is the same for all mammals, although the size varies. The overall anatomy of the eye is shown in cross section in Figures 1 and 2 (Wolff, 1968). Major tissues and fluids include cornea, iris, ciliary body (includes ciliary processes and ciliary muscle), aqueous humor and vitreous body, retina, and lens.

The lens of the eye is a transparent body of crystalline appearance placed between the iris and the vitreous body. The human lens is a transparent, biconvex, semi-solid body devoid of any blood supply (ICRP #23, 1975). The adult lens has a diameter of about 9-10 mm and a thickness of



A.O. = optic axis
C.v. = vitreous body
M.c. = ciliary muscle
z. = zonule
F.c. = focal spot
L. = lens
C.b.a. = aqueous humor
j. = iris

Figure 1. Cross Section of the Eye (Wolff, 1968)

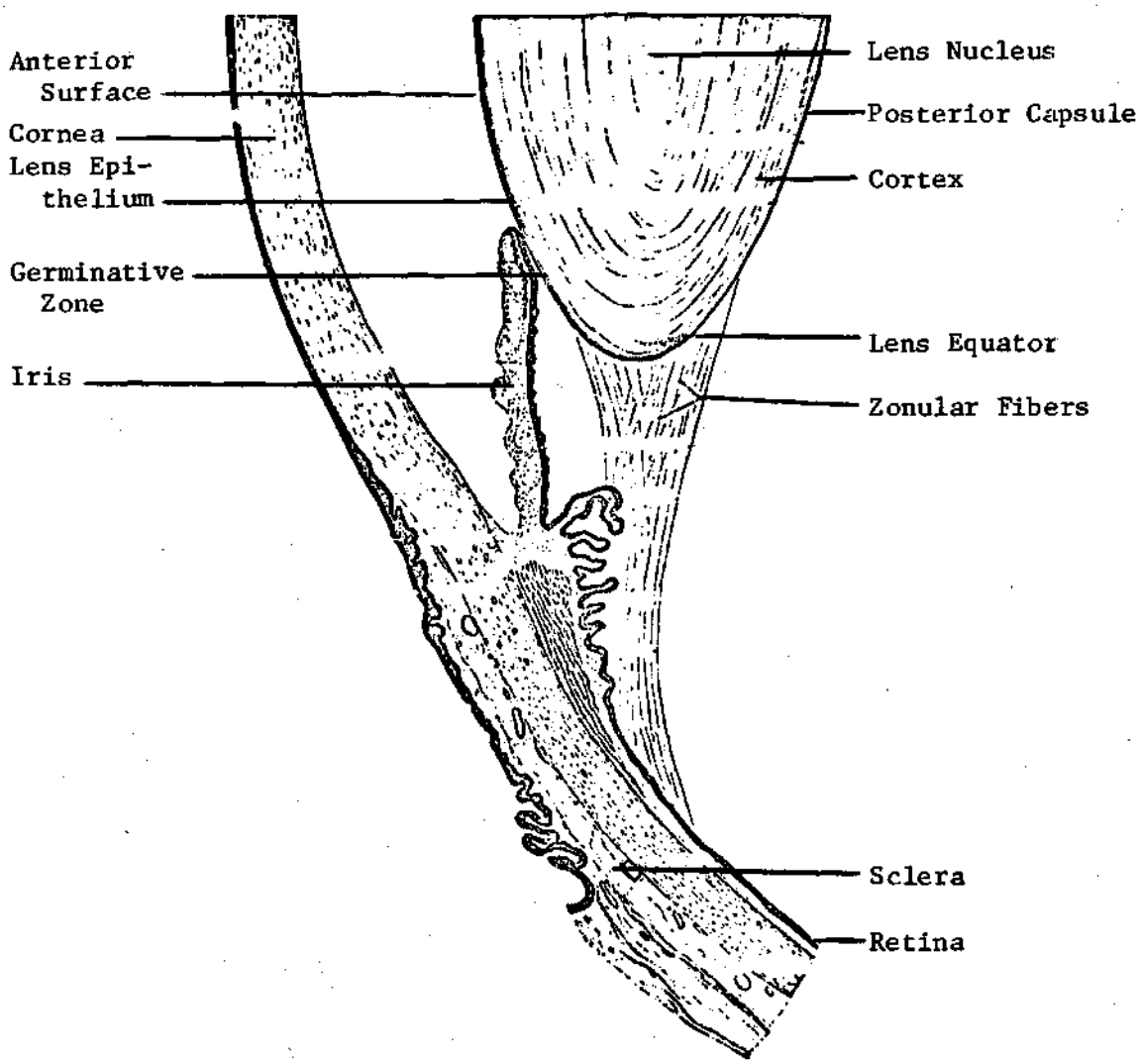


Figure 2. Detail of Cornea and Lens (Wolff, 1968)

4-5 mm. The anterior surface is termed the anterior pole, while the posterior surface is called the posterior pole. The junction of the two poles defines the lens equator. The lens of the eye is suspended by ligaments extending from the ciliary muscle and undergoes changes of shape or accommodation by means of the contractile state of this muscle. This results in an increased curvature of the anterior pole based on two factors: (1) the malleability of the lens matter, and (2) the elasticity of the anterior lens capsule. Maximum accommodation occurs at about eight years of age (14 diopters) and decreases with age to about one to two diopters in the 50-60 age group.

The weight and volume of the lens gradually increases throughout life. The total weight of both lenses for the reference adult male and female is about 400 mg.

The lens is entirely surrounded and enclosed by a capsule. The thickness of the anterior capsule is 0.011 - 0.018 mm and that of the posterior capsule, 0.005 - 0.007 mm. The capsule is composed of a noncellular membrane mainly secreted by the lens epithelium with an overlying thin zonular layer derived from the zonular fibers, i.e. a single layer of epithelial cells on the anterior surface just under the capsule. The lens epithelium is composed of the central zone, the germinative zone, preequatorial, and equatorial zones. The posterior capsule is much thinner than the anterior capsule and there is no epithelial cell layer under it.

The capsule is largely composed of a single major constituent, an insoluble protein containing about 10% carbohydrate. The capsule contains 14% nitrogen, 9-10% carbohydrate and about 1% hexosamine. No phosphorus

is present. Sodium and potassium enter and leave the lens through the aqueous humor. Sodium and potassium are two of the most important electrolytes in living cells and they provide inorganic chemicals for cellular reactions. Also, they are necessary for operation of some of the cellular control mechanisms such as transmission of electrochemical impulses in nerve and muscle fibers and the activity of different enzymatically catalyzed reactions necessary for cellular metabolism (Guyton, 1971).

Within the capsule, excluding the anterior epithelial layer, the lens consists of highly organized, parallel fiber bundles extending from front to back and transverse to the long, equatorial axis. The composition of these fibers is given in Table 1.

Table 1. Chemical Composition of Adult Human Lens

Substance	Percent of Lens Wet Weight
Water	62.6
Protein	35.5
Lipids	1.7 - 2.3
Ash	0.068 - 0.73
RNA	0.058

NOTE: Table from ICRP #23, 1975.

The interior of the lens is divided into cortex and nucleus. The cortex lies just under the capsule and is the region of newest fibers. The cortex increases from 0.4 mm at 20 years of age to 0.8 mm at 80 years of age.

The thickness of the anterior cortex increases approximately 0.007 mm per year. As one moves from the cortex deeper into the lens nucleus, older and older fibers are crossed until the oldest tissue is found at the center of the lens. The nucleus of the lens is the central region and has a mean thickness of about 2.8 mm with a range of 2.2 - 3.6 mm.

The lens grows from a central embryonic nucleus by adding fibers much like a tree adds rings throughout its life. The adult lens grows very slowly and is almost a resting tissue. This slow growth rate gradually adds weight and volume to the entire lens.

The increasing density of the nucleus with increasing age is the result of loss of water and compression of the lens fibers. As the formation of new fibers takes place, the older fibers are compressed and pushed in toward the center. This results in increasing density of the lens from the surface to the center. This increasing density and water loss from the lens alters or partially inhibits accommodation and may change the refractive index of the lens. Thus, the lens hardens with age and is less able to change its shape and focus light. The chemical composition of the lens is also presented in Table 1.

The average water content of the whole lens is about 63%. There is a greater proportion of water (up to 75% by wet weight) in the cortex due to the fact that its protein content is composed of the soluble (in water) crystallin proteins. The average water content of the standard man is 60% (ICRP #23, 1975).

The lens contains a higher percentage of protein than any other organ in the body. The total amount of protein is about 35% of the lens

mass, compared to a value of 15% for the entire body (ICRP #23, 1975). The lens nucleus contains a higher percentage of the total protein than the cortex, depending on the age of the individual.

The principal trace elements in the lens are presented in Table 2.

Table 2. Concentrations (mg ion per 100 g lens wet weight) of Inorganic Ions in Adult Lens

Ion	Man	Cattle	Rabbit	Rat
K	170.0	404.0	472.0	222.0
Na	91.0	46.0	59.0	78.0
Cl	35.3	69.0	35.0	29.0
Ca	1.4	6.0	1.85	5.0
Mg	0.29	8.0	0.58	--
SO_4	--	468.0	--	2.4
PO_4	--	6.2	4.3	24.0
CO_2	--	--	--	2.3

NOTE: Table from Kuck, 1970.

Sodium is found in the extracellular fluid whereas potassium is found inside the fibers. In the absence of calcium, cell membrane permeability is increased. The potassium content of the lens is very high, many times higher than that of any other tissue of the eye. Once inside the cell, potassium combines with other substances and becomes impermeable to the cell membrane.

As the lens ages calcium deposition increases and in old lenses, calcium is present in considerable amounts.

The significance of the high Mg and SO_4 values in the cow lens is not readily apparent but the function of magnesium in the lens is almost certainly connected with its capacity to act as a co-factor in several enzymatic reactions (Kuck, 1970).

Lens Physiology

The energy requirements of the normal lens are limited to the maintenance of its transparency, nourishment for the development and growth of new fibers, and the maintenance of capsule elasticity. The lens grows very slowly but continuously throughout life without being able to slough off old cells because of the lens capsule. No new cells can enter the lens nor can old cells be discharged as such.

Glutathione plays an important role in the physiology of the normal lens (Adler, 1965). In nearly all forms of cataract, including radiation cataracts, it tends to disappear from the lens. Glutathione consists of the amino-acids glycine, cysteine and glutamic acid. The cysteine fraction, containing a sulfhydryl group (-SH), is the most reactive constituent and enables glutathione to exist in two forms, oxidized S-S and reduced -SH. Reduced glutathione contains cysteine, whereas oxidized glutathione contains cystine. Injection of cysteine into the eye before exposure to ionizing radiation exerts a protective effect on the lens (von Sallmann, 1952). Cysteine administered intravenously to rabbits 30 minutes prior to irradiation with x-rays prolonged the inhibitory effects of x-rays on mitosis in the lens epithelium, slowed the speed of recovery and reduced the

extent of overshooting of cell division, for exposures of 1,500 R to the eye.

The protective effect of pretreatment with cysteine against x-ray damage of the lens was demonstrated by a lower incidence of cell nuclear fragmentation in irradiated lenses as compared with the incidence of nuclear fragmentation in the lenses of untreated irradiated rabbits and of rabbits treated after irradiation (von Sallmann, 1952).

The amount of oxygen which the aqueous humor supplies to the lens is relatively small and is comparable to the amount of oxygen physically dissolved in most body fluids. The aqueous humor itself has a normal oxygen tension of 40-50 torr. The rabbit lens uses about 0.25 mg of oxygen per day. Almost the entire amount of oxygen utilization is by the lens epithelium and cortex. The utilization of oxygen by the nucleus decreases with age because of the decreased metabolic activity of all older tissues. The lens capsule and the nucleus of both bovine and rabbit lenses consume negligible amounts of oxygen. Since the oxygen uptake rate is one measure of the metabolic activity of tissue, the low rate of oxygen uptake by the lens indicates a rather sluggish respiration compared with other tissues.

Carbohydrates are the major energy substrates for the energy requirements of the lens. When oxygen is present, glucose may be completely broken down to carbon dioxide and water. In the absence of oxygen, glucose can be converted only into lactic acid by a series of complex steps.

Prominent among the physical changes in the lens which is aging is the sclerosis of the inner fibers and a reduction in potassium. Sodium and chloride increase as the processes fail whose function it is to keep them

out (Kuck, 1970). The potassium concentration in the whole lens is only slightly reduced in aging (Kuck, 1970). A slight but probably significant rise in sodium concentration leads to a decrease in the K/Na ratio in the ox lens from 7.0 to 5.7. In aging the cow lens accumulates sodium at a level which is 117% of that found in the calf lens; the potassium level falls to 96% of that in the calf lens.

The levels of sodium, potassium and calcium in the rat lens have been shown to decrease asymptotically with aging when the values are expressed as microequivalent ions per mg lens wet weight (Kuck, 1970). The concentration of these ions per unit weight or per unit volume was found to become nearly constant after about 100 days post-fertilization in the rat.

CHAPTER II

GENERAL RADIATION EFFECTS ON OCULAR STRUCTURES

Before discussing the pathogenesis of radiation cataractogenesis in the mammalian lens, it is appropriate to cover briefly those effects of ionizing and non-ionizing radiations on the other important tissues of the eye. The information to date concerning ionizing radiation cataracts in the lens of the eye is of primary concern in this work and will be covered at length in a later chapter.

According to Draper's law (1840) only the energy which is absorbed by a system can affect it (Cogan, 1950). With the exception of cosmic rays and very long radiofrequency waves (e.g. radio and TV), which readily pass through the body, there is no type of radiation which has not been found at some time to have induced an ocular lesion. Long radio-diathermy waves are transmitted through the eye readily without absorption and are commonly considered to produce little biological effect. Short diathermy waves, those with wavelengths of the order of several centimeters, induce heat effects in the eye with harmful effect on the anterior segment of the eye. Long infrared rays are absorbed almost entirely by the cornea and aqueous humor; the short infrared, visible and near ultraviolet rays are transmitted to the retina.

Most of the absorption of ultraviolet light by the lens occurs between 400 and 295 millimicrons but below 295 millimicrons absorption is chiefly by the cornea. In fact the cornea is almost opaque to wavelengths

shorter than 293 millimicrons. Of the softest grenz rays (8-25 kV), 97% are absorbed by the cornea, of the hardest, 11%. Grenz rays are capable of inducing cataracts but corneal damage would result if these rays were used as cataractogenic agents (Lerman, 1962). At 110-200 kV, 90% of the x-ray energy incident on the anterior surface of the adult human cornea will reach the retina (Duke-Elder and MacFaul, 1972).

The infrared effects on the cornea are simple thermal burns appearing immediately after exposure and resulting in an opacification of the cornea through coagulation of the protein. The effects of UV light on the cornea include burns resulting from welder's arcs (flash eye) and quartz mercury vapor lamps. Keratitis resulting from UV radiation from the sun also occurs with prolonged exposure at high altitudes or in the presence of large snow fields. Symptoms of keratitis come on after a latent period of 5-12 hours.

Of the energy reaching the earth from the sun about 39% is in the visible range and 55% in the infrared. The intensity of the sun at sea level on a clear day with the sun at the zenith is 1.72 calories per cm^2 per minute. The amount of energy necessary to produce a retinal lesion in rabbits with a spectral distribution corresponding to that of the sun is 50 calories per cm^2 per min on the retina for an exposure of 30 seconds, and 100 calories per cm^2 per minute for exposures of several seconds, the amount depending on the size of the image.

The conjunctiva exhibits symblepharon (adhesion of the lids to the globe) after doses of 8,000 to 10,000 rads of 200 kVp x-rays. The iris is more sensitive; rubeosis iritis (vascularization) can occur after 1000 to 2000 rads. The cornea, normally avascular, is also more sensitive than

the conjunctiva. Keratitis may follow 5,000 R and corneal ulceration after 10,000 R of 200 kVp x-rays (Wachtler, 1961). Edema, perforation and hypoesthesis also occur. X-rays are often used to treat neovascularization in the cornea due to a phenomenon known as the indirect vascular reaction. In vascularized tissues the action on the blood vessels is the most potent factor determining tissue death after irradiation. There is vacuolar degeneration of the cells of blood vessels, enormous dilation and engorgement, thrombus formation and stoppage of circulation. Constrictions alternate with dilations (Duke-Elder and MacFaul, 1972).

The human sclera has a poor blood supply and is highly radioresistant--doses up to 40,000 R have been tolerated without injury (Williams, 1967). The retina, being more like cerebral tissue than systemic tissue, is also very radioresistant. The blood vessels of the retina behave as cerebral vessels and are resistant in the adult mammal. However, the retina of young animals is very sensitive to radiational mitotic inhibition. Doses of 3500 R of 200 kVp x-rays are well tolerated by the retina (Duke-Elder and MacFaul, 1972). Above 10,000 R functional disturbances are evident and at 30,000 R there is severe injury to the ganglion cells (Wachtler, 1961). The retina has tolerated doses up to 1200 rads in four weeks with Co-60 applicators with only slight pinkness resulting (Williams, 1967).

The human aqueous humor and vitreous body respond similarly to radiation. Exposure to 1000-2000 R of x-rays or gamma radiation will cause partial liquefaction of the vitreous gel. Cells appear in the vitreous body following exposure and the intra-ocular pressure may rise of fall. Tables 3 and 4 present a summary of the effects of ionizing radiations

Table 3. Effects of 200-250 kVp X-Rays on the Adult Human Eye

Tissue	Effect	Latent period		Dose, rads	
		Early	Late	Single	Divided
Lid skin	Erythema	2-4 weeks		600	$ED_{50} \sim 600\ t^{0.33}$ (daily schedules)
	Pigmentation	2-3 weeks		400-600	
	Depigmentation		6-12 months		4,000-5,000/4-5 weeks
	Telangiectasis		2-5 years		3,000-5,000/3-5 weeks
	Moist desquamation	2-8 weeks			5,000-6,000/5-6 weeks
	Scarring		> 6 months		8,000/3-8 weeks
Lid tarsus	Atropy (thinning)		> 6 months		4,000-5,000/4-5 weeks
Lid margin	Epilation (incomplete)	1-2 weeks		1,000	
	Epilation (complete)	2-5 weeks			2,000-3,000/2-3 weeks
	Rounding of		6-12 months		4,000-5,000/4-5 weeks
	Ectropion, Entropion		> 1 year		8,000-10,000/1-4 days
Lacrimal gland	Atrophy		> 6 months	2,000	5,000-6,000/5-6 weeks
Conjunctiva	Hyperemia	immediate		> 500	
	Conjunctivitis	1-3 weeks			~ 5,000/4-5 weeks
	Telangiectasis		2-5 years		3,000-5,000/3-5 weeks
	Keratinization		> 1 year		5,000-10,000/5-10 weeks
	Symblepharon		> 1 year		8,000-10,000/1-4 days

NOTE: Taken from Merriam, 1972.

Table 4. Effects of 200-250 kVp X-Rays on the Adult Human Eye

Tissue	Effect	Latent period		Dose, rads	
		Early	Late	Single	Divided
Cornea	Punctate keratitis	several weeks		1,000	3,000-5,000/4-5 weeks
	Edema	1-3 weeks			4,000-5,000/2-3 weeks
	Mild ulceration	several weeks			3,000-4,000/2-3 weeks
	Chronic ulceration		several months	~ 2,000	$\gtrsim$ 6,000/5-6 weeks
	Perforation		4-12 months		$\gtrsim$ 6,000/5-6 weeks
	Thinning		several months		3,000-5,000/3-5 weeks
	Scarring		~ year		$\gtrsim$ 6,000/5-6 weeks
	Keratinization		~ year		$\gtrsim$ 5,000/4-5 weeks
	Vascularization		> year		$\gtrsim$ 5,000/2-3 weeks
	Lipid infiltration		> year		$\gtrsim$ 7,000/5-7 weeks
	Poor wound healing				$\gtrsim$ 18,000
Sclera	Atrophy		several years		20,000-30,000
Iris	Iritis	several days		2,000	$\gtrsim$ 6,000/5-6 weeks
	Vascularization (rubeosis)		several months		7,000-8,000/6-8 weeks
	Atrophy		> 3 years		17,000-25,000
Lens	Cataract		> year (1-20 years)	$\gtrsim$ 200	$CD_{50} \sim 400\ t^{0.17}$ (daily schedules)
Retina	Edema	several weeks			2,000-3,500 in 3-4 weeks
	Vascular occlusion		6 months to 3 years		
	Telangiectasis				
	Hemorrhages				3,000-5,000 in 3-5 weeks
	Exudates				

Table 4. Continued

Tissue	Effect	Latent period		Dose, rads	
		Early	Late	Single	Divided
Retina cont.	Degeneration			experimental	
	rods			2,000	
	cones			> 10,000	
	ganglion cells			~ 30,000	
	Atrophy				5,000-8,000/4-8 weeks
Orbit	Growth failure		several years		4,000-7,000/3-7 weeks

NOTE: Taken from Merriam, 1972.

on human ocular structures.

Ocular malformations due to radiation-induced genetic mutations have been demonstrated by McGregor and Newcombe (1972). Low doses of ionizing radiation (200 kVp x-rays) in the range from 25-400 rad to mature trout sperm resulted in major eye malformations in the immediate offspring. About 54 rad was required to double the rate of malformations observed in the controls. It was concluded that the malformations were due to chromosome aberrations induced in the mature sperm.

In summary the various components of the eye may be listed as follows in order of increasing radioresistance: lens, cornea, conjunctiva, aqueous and vitreous humors, sclera, and retina. In cases of ionizing radiation exposure, it is the lens, therefore, which is the critical tissue in the eye and it is to this component that we now focus our attention.

CHAPTER III

PATHOGENESIS OF CATARACTS INDUCED BY IONIZING RADIATION

The term cataract is used loosely to mean the occurrence of an optical discontinuity in the lens of such magnitude as to cause a noticeable dispersion of light (Kuck, 1970). Accumulations of other partly opaque substances, such as crystals of calcium salts, are considered lens opacities, reserving the term cataract for changes primarily involving lenticular protein. Cataracts are symptomatic and are the end-products of many insults to the lens. It appears highly likely that all pre-cataractous changes have much in common so that information obtainable from experimental cataractogenesis may be pertinent to the development of common types of cataract in man, namely senile cataracts (Kuck, 1970).

Possible Mechanisms of Cataract Formation

Several theories of the etiology of radiation cataracts have been proposed (Iowa State University, 1961): (1) Cataracts may be caused by primary radiation injury to the lens epithelium, (2) The lens fibers are the site of primary radiation damage, (3) Cataract may be the result of secondary damage to the lens due to primary injury to adjacent tissues that nourish the lens such as the aqueous humor or the ciliary body. It is almost certain that any indirect effect due to whole-body irradiation is of no major importance in radiation cataract or, for that matter, any other ophthalmic lesion (Upton, Christenberry and Furth, 1953; Lorenz and Dunn,

1950; Poppe, 1957). The response of the lens to ionizing radiation, therefore, is the same whether the entire animal is exposed or only the lens.

The theory that cataracts may be caused by primary radiation injury to the lens epithelium is based on the observation that, of all the sectors of the entire lens, it is the most mitotically active. An indication of the relative mitotic activities of the four epithelial segments has been given by Riley and Devi (1965). The following listing gives the mitotic indexes of the central, germinative, pre-equatorial and equatorial zones of the lens epithelium:

Epithelial Zone	Mitotic Index (fraction of dividing cells/1000 cells)
Central	0.00105
Germinative	0.00337
Pre-equatorial	0.00131
Equatorial	0.00000

Riley (1967) used tritiated thymidine labeling to determine the residence time of cells in the epithelium. The cell population turnover time in the central zone of the mouse lens was found to be 198 days and in the peripheral zone 23 days. Riley postulated that most of the cells in the central zone (perhaps 98%) are in a G_o compartment, i.e. a stage that is metabolically active and not proliferating, but retaining the capacity to proliferate. Wounding may stimulate these G_o cells to shift into the proliferating compartment of the cell cycle. Cells in the peripheral region are in one of three compartments: the G_o, the proliferating, or the differentiating compartment. All of the cells in the equatorial

region appear to be in the differentiating compartment. The long turnover time calculated for the population in the central zone could be accounted for by the large proportion of cells in the G_o compartment.

Hanna and O'Brien (1963) have postulated a different theory as to which cells in the lens are the site of the primary radiation damage. Work with mice and rats exposed to 2400 R of Co-60 gamma radiation seemed to indicate that damage to the newly forming lens fibers and not the undifferentiated germinative cells leads directly to the cataract. Tritiated thymidine was used to label cells synthesizing DNA and was injected into the anterior chamber just after exposure and prior to enucleation. The labeled cells were followed until they formed fibers and lost their nuclei. In the adult rat these labeled cells were not found to enter the lens cortex until after the cataract was well advanced or complete. In younger animals cell migration was fast enough so that in some cases labeled cells moved into the lens and migrated toward the posterior pole before the cataract was complete. In the mouse these labeled cells migrated toward the posterior pole just under the lens capsule to form opacities. Lens fiber damage in all cases was well advanced before cells in the zone of DNA synthesis at the time of irradiation had migrated and reached the area of opacity in the lens cortex.

Young, damaged nucleated fibers appeared to be the first constituents of the opacity at the posterior pole. The nuclei of these fibers moved toward the posterior pole whereas normal nuclei movement is toward the anterior pole. Later, opacities formed at the posterior pole from damaged epithelial cells which were irradiated in the germinative zone.

Whichever theory is correct, it is certain that radiation first damaged nucleated cells as opposed to non-nucleated cells. Radiation cataracts are generally cortical in type, whereas some senile cataracts in man are nuclear cataracts.

Time Sequence or Acute vs Delayed Effects in Cataract Formation

The latency period (several months to years) observed between the initial radiation injury and the clinical appearance of opacity as observed by means of a slit-lamp or the ophthalmoscope is postulated to be the time taken for the damaged epithelial cells in the germinative zone to migrate to the equator where they then differentiate into abnormal lens fibers (Vogel, 1973). These abnormal fibers are then pushed toward the posterior pole of the lens where they constitute the incipient opacity. Depending on the dose and quality of the radiation received, the entire lens may become cloudy and vision destroyed.

Hanna (1961) used tritiated thymidine labeling to study cell migration in the epithelial cell layer. Tritiated thymidine was injected into the anterior chamber of the eye of mice and rats. Cell movement was followed using autoradiographic film techniques on histological sections of the lens.

In the newborn rat the labeled epithelial cells were scattered over the entire anterior surface of the lens with some concentration occurring in the equatorial region. As the rats became older the labeled cells were increasingly concentrated in the pre-equatorial zone. The labeled epithelial cells were followed from the germinative zone into the lens up to the

point where the growing fibers extruded their nuclei anteriorly. The amount of time used by the cells to move this distance varied from an estimated two months when the rats were 17 days old to an estimated four months in the two month old rat. By the time the rats were five months old the lens epithelial cell migration rate had slowed considerably, taking two months to go just one quarter of the above distance. Cell movement in the ten month old rat was similar to that in the five month old rat, leading to a slow but continuous formation of new lens fibers in adult mammals. Similar results have been obtained in the mouse.

A number of experiments have been performed with rabbits exposed to 1500 R of 200-250 kVp x-rays to study lens cytopathology and the sequence of events in the lens leading to cataract formation (Poppe, 1942; Wanko, 1959; von Sallmann, 1952). Nuclear fragmentation of the epithelial lens cells and extrusion of clumped chromatin material into the cytoplasm begins two hours after irradiation. Such signs are clearly evident 24 hours post-exposure. Cells in the equatorial zone are depleted; this is interpreted to be the result of nuclear fragmentation which leads to liquefaction of the cell bodies with vacuole formation. In general the frequency of nuclear fragmentation is greatest in the germinative zone. Here the cells have the highest content of DNA. The fragmentation rate was also high in the pre-equatorial zone but relatively low in the large central zone. The degenerate cell count at two weeks after exposure rises steeply, at which time cell division is in an overcompensating phase (the mitotic activity may be as much as 180% of controls).

Twenty-four hours after irradiation obvious changes in nuclear structures are evident, mainly involving the location of nucleoli. Normal

nucleoli are often eccentrically located but well separated from the nuclear envelope by karyoplasm. In irradiated cells they are frequently seen at the periphery, immediately adjoining the nuclear envelope. On the fourth day post-exposure polynucleated cells are observed in the equatorial area. Sometimes as many as four small nuclei may be observed in a single cell.

Cells affected by the radiation are randomly distributed throughout the epithelium but are much more numerous in the equatorial zone. Changes occur in all cell constituents except the Golgi complex and range from slight alterations of individual components to partial disintegration of the entire cells. These lesions gradually increase with time after irradiation.

One month following an acute exposure of 1500 R of 200 kVp x-rays the first lens fiber changes are noticed. These changes appear in the equatorial region as swollen fibers with vacuolar formation. Some of the pathologic fibers posterior to the equator are nucleus-containing and are called pseudoepithelium. Four months after exposure the cataract first becomes clinically evident and appears as numerous opacities peripherally located in the posterior cortex. At 14 months post-exposure there is pronounced polymorphism of cell nuclei with the formation of giant cells, some containing multiple nuclei and several accessory nuclei. At this time many cells in the central epithelial zone appear abnormal as well.

Radiation Doses Required to Form Cataracts

A single, acute exposure of 2000 rads of 200 kVp x-rays will induce cataracts in young rats in about 50% of the irradiated animals within 10 weeks after exposure (Focht, 1966). Moderately advanced cataracts will be observed in all irradiated rats at about 20 weeks post-exposure. If the same total dose is divided into three equal fractions given over a six day period, the onset of cataract in rats may be delayed as much as ten weeks beyond the latency period observed for single exposures.

The above dose required for cataract formation is greatly reduced if only minor (not interfering with vision) lens changes are considered. For example, the lowest single exposure for the production of lens changes in mice which are detectable by skilled examination only is about 33 R of 200 kVp x-rays and about 1.3 rads of fission neutrons (Vogel, 1973).

An interesting footnote to the pathogenesis of radiation cataract in mammals is the complete immunity of the bird lens to radiation cataract (Pirie, 1959). Doses as high as 6000 R of 200 kVp x-rays do not produce cataracts in the lens of the chick embryo. The lens of the bird differs from that of the mammal in that there is a lens pad of several layers of epithelial cells situated as a peripheral ring just at the lens equator. If the chick embryo is irradiated, the lens epithelium shows immediate damage but the degenerate cells are lysed and within a few days it is difficult to see more than traces of damage. The essential difference lies in the fact that the chick lens rids itself of damaged cells completely, whereas in mammals the cells differentiate to produce abnormal lens fibers. The mammalian lens retains its damaged cell population for the life of the animal.

Summarizing, the initial injury which eventually leads to cataract is an insult to the epithelial cells just under the lens capsule near the equator. It is these cells which are most sensitive to ionizing radiation and the latency period between irradiation and the initial onset of opacity appears to be the time taken by these damaged cells to migrate to the posterior lens pole as abnormal, opaque fibers. Thus, the actual cataract is a late radiation effect which is the end result of many previous biochemical lesions in the lens. It is these biochemical insults and alterations in lens chemical composition prior to the onset of opacity which are of major concern in this dissertation.

CHAPTER IV

SURVEY OF THE LITERATURE ON IONIZING RADIATION CATARACTOGENESIS

Radiation Cataractogenesis in Experimental Animals

There is a substantial amount of information regarding experimental radiation cataractogenesis in laboratory animals. In view of this it seems appropriate to cover these investigations separately from ionizing radiation effects on the human lens. Ionizing radiation cataractogenesis in animals will be discussed under the following sub-headings: x-rays and gamma rays in order of ascending energy, neutrons in order of increasing energy, protons and other heavy ions, and beta rays. In addition some consideration will be given to relevant topics such as differential species sensitivity to cataracts induced by ionizing radiation, the oxygen effect, lens protection, fractionation and protraction of dose, and radiation aging of the lens.

Experiments with Low-LET Radiation

In his classic work on the eye, the well-known System of Ophthalmology (1972), Sir Stewart Duke-Elder traces in his volume on non-mechanical injuries the history of the early researches on the effects of x-rays on the crystalline lens (Duke-Elder and MacFaul, 1972). About 1905 to 1907 von Hippel demonstrated that no tissue of the eye shows a greater sensitivity to ionizing radiation than the developing lens. Of the many ocular

anomalies he found in his early experiments on the x-irradiation of the pregnant uterus of rabbits, cataractous changes in the lens were the most common.

Further experiments by Tribondeau and Recamier in 1905, Belley in 1907, Tribondeau and Belley in 1907, Bossuet in 1909, Froge in 1922 and Lorenz and Dunn in 1950 showed that the same susceptibility is seen in the lens of the young and rapidly growing animal (Duke-Elder and MacFaul, 1972). The changes described by these authors were classic examples of ionizing radiation cataracts--the subcapsular epithelial cells in the equatorial zone of the lens were first destroyed, the lens fibers in this region became swollen and tortuous, and about the 33rd day after irradiation lenticular opacities became clinically evident, leading to the rapid development of cataract.

Early radiation workers who studied the adult lens concluded that it was insensitive to x-rays because of the long latent period (several months to years) which exists before cataractous changes appear in older lenses. They simply did not wait long enough to see pathological changes. These early preliminary experiments included those of Chalupecky during the years 1897-1911, Scholtz in 1902, Birch-Hirschfeld in 1904, Tribondeau and Lafargue in 1907-1908, Rauch in 1914, Rollet and Malot in 1914, Rados and Schinz in 1922 and Jacoby in 1924. It was not until the appearance of the work of Aulamo during the period 1928-1931 and particularly that of Rohrschneider from 1929 to 1939, both on the rabbit, that the cataractogenic properties of x-radiation in adult experimental animals were recognized. Although the adult lens is less sensitive to ionizing radiation and takes

longer to develop opacities, pathological changes are essentially the same as in the young lens.

Investigations that followed these first definitive studies confirmed the earlier work and included those of Goldman in 1929 on the rat and Kleiber in 1930 on guinea pigs.

Kandori (1956) conducted studies with rabbits to determine the minimum cataractogenic dose of 80 kV photons. He found that an acute dose of 1,000 R of 80 kV x-rays produced cataracts in 50% of exposed animals within one year after irradiation. No lens changes occurred after administering fractionated doses of 15 R daily for 185 days; after giving 60 R per day for 130 days, however, histological changes were discovered 261 days after commencement of the exposures.

A study of the effects of 124 kV x-rays on the incidence of lens opacities in mice is reported by Clark and Sykowski (1952). Approximately 50 weeks after an exposure of 200 R at 124 kV, cataracts occurred in 80% of the exposed mice.

Riley and co-workers (1957) exposed mice to 250 kVp x-rays to study fractionation effects in that animal. The cataractogenic effectiveness of x-rays decreased as smaller, more frequent doses were administered (nine doses of 133 rad each were less effective than three doses of 400 rad each). Fractionated doses of x-radiation were considerably less cataractogenic than a single dose of the same total amount (five doses of 200 rad each were equivalent to a single dose of about 550 rad).

Focht (1964) found an RBE of one for Co-60 gamma rays compared to 200 kV x-rays for cataractogenesis. She used six month old rats in her study. The LET of 200 kV x-rays is on the average 1.8 keV/micron in water,

whereas the mean LET of Co-60 gamma rays is 0.27 keV/micron in water. A slightly different result is indicated by Upton et al. (1956). For mice irradiated at high dose levels and at high intensity by x-rays and gamma rays, 250 kVp x-rays appeared to be slightly more cataractogenic than gamma rays (RBE = 1.2).

The cataractogenic effects of 50 MeV photons on the mouse lens have been investigated by Clark and Sykowski (1952). The 50 MeV x-rays were obtained from a betatron from which the neutron dose was considered to be insignificant in comparison with the x-ray dosage. Induced radioactivity from the 50 MeV x-rays amounted to only 0.005 rad in tissue from a dose of 3,050 rad. A dose of 200 R produced cataracts in 70% of the exposed mice at one year post-exposure. A dose of 400 R induced cataracts in 95% of the animals one year after irradiation.

Experiments with Neutrons

The first observation of the cataractogenic property of neutrons was made by Horn (1941) on larvae of the Amblyoma, a large tick. Since that time it has been recognized that there are vast differences between lens damage due to fast neutrons and that due to radiations of lower LET. The chief differences between cataracts produced by neutrons of all energies and those produced by x-rays are: (a) A shorter latent period for development of the opacity is seen with neutrons as opposed to that observed after equal doses of x-rays. (b) A larger dose of x-rays is needed to produce the same effect, i.e., the relative biological effectiveness or RBE of fast neutrons is greater than unity. (c) Neutrons have greater specific ionization than x-rays. This minimizes the amount of tissue repair which

may occur after irradiation and results in an increasing RBE with dose protraction. (d) Neutrons seem to have a greater effect on resting cells, causing primary damage. In neutron cataracts primary damage to the lens fibers is found concurrently with damage to the epithelial cells.

The cataractogenic properties of thermal neutrons from the thermal column of the Los Angeles homogeneous reactor have been studied by Storer and Harris (1952). The incidence of lens opacities in Swiss mice was significantly higher after exposure to mixed radiation from the thermal column as compared to the incidence of cataracts after exposure to 250 kVp x-rays. The RBE of thermal neutrons was found to be 15 with regard to production of lens opacities and was nine times the RBE based on lethality at 30 days. In contrast, Upton et al. (1956) found a thermal neutron RBE in the neighborhood of three to six for mice given a single, brief exposure.

A number of experiments have been conducted to investigate the effects of fission neutrons on the lens. Cogan et al. (1952) studied the production of cataracts in rabbits from single exposures to fission neutrons. The threshold dose for production of lens opacities by fission neutrons was found to be less than $2 \cdot 10^9$ n/cm^2 incident on the lens. These same researchers found fission neutrons to be comparable to 14 MeV neutrons for cataract production in another experiment (Cogan et al., 1953). The energy released at the cataractogenic level by these two neutron groups of widely different energies was of the same order of magnitude (i.e., $4.2 \cdot 10^3$ erg/cc for 14 MeV neutrons and $5.4 \cdot 10^3$ erg/cc for neutrons of fission energy).

Monoenergetic neutrons of 0.43, 1.00, 1.50 and 1.80 MeV were used

by Merriam et al. (1965) to study the dependence of RBE on the energy of fast neutrons for the induction of lens opacities in mice. The mice were also irradiated with Co-60 gamma rays. Gamma ray dosimetry was accomplished using a Victoreen thimble ionization chamber placed within the mouse exposure box. It was assumed that the absorbed dose in rads received by the lens of the eye is equal to the exposure thus determined in rads multiplied by a factor of 0.96. Neutron dosimetry was carried out with a tissue-equivalent ionization chamber filled with tissue-equivalent gas. Results indicated that in general the RBE increases from about 4.5 to about 9 as neutron energy decreases from 1.8 to 0.43 MeV. On the other hand, Bateman et al. (1963) found that neutrons of 0.43 and 1.80 MeV are about 10 times more effective than x-rays at all doses for lens opacification in mice, with the 0.43 MeV neutrons slightly more effective than those of 1.8 MeV. One rad of neutrons at either energy appeared to produce definite opacification, with no evidence of the opacity becoming stationary, up to 32 weeks after exposure.

Neutrons in the energy range from 6-8 MeV were studied by Moses et al. (1953) to determine cataractogenic effectiveness in dogs. One exposure of 120-300 rads failed to produce cataracts in the animals over a two year period. A dose of 1,620-1,800 rads produced cataracts in 60-75% of adult dogs.

Riley et al. (1955) have investigated the relative cataractogenic effectiveness of fast neutrons from 1-12 MeV. The RBE appeared to vary from 2.8 for neutrons of about one MeV to 1.2 for neutrons in the 7-12 MeV range. However, even at the higher energies the fast neutrons produced

a complete cataract at exposures well below the LD-50, which was not the case for x-rays. Fractionation decreased the cataractogenic effectiveness of x-radiation far more than it did for the neutrons.

Fourteen MeV neutrons from the T(d,n)He reaction are a favorite tool of investigators in radiobiology. Choshi (1964) found that, histologically speaking, cataracts induced by 14 MeV neutrons are similar to those induced by x-rays; there was no qualitative difference. In similar studies Choshi (1963) found that adult rabbits irradiated with 14 MeV neutrons showed typical formation of posterior subcapsular cataract with degeneration of the anterior subcapsular epithelium at the region of the equator. The threshold dose for permanent damage and loss of vision appeared to be $3.2 \cdot 10^{10}$ n/cm^2 incident on the lens. In lenticular damage studies with rabbits irradiated with 14 MeV neutrons, Hosokawa (1967) found an RBE of 14 as compared to 180 kV x-rays at doses from 100-180 R. The RBE of 14 corresponded to minor lens opacities not interfering with vision. For permanent lens opacities resulting in impaired vision, the RBE was 3.5.

At least one study investigated the cataractogenic effects of 15 MeV neutrons on six month old rabbits (Graul et al., 1968). In this experiment the neutron RBE with respect to Co-60 gamma rays was found to be two for single acute exposures of 750 rad of neutrons and 1,500 rad of gamma rays.

Cataracts Induced by Protons and Heavy Ions

Ophthalmologic studies using charged particle beams are scarce, particularly in the mouse. A comparison of 60 MeV protons and 300 kVp

x-rays for induction of lens opacities in RF mice was conducted by Darden and associates (1970). In a study of the relative biological effectiveness of protons for late somatic effects in mammals, eight week old female mice either received whole-body 60 MeV proton or 300 kVp x-radiation in single graded doses up to 400 R or were sham exposed. Results showed that for 35-60 MeV protons the RBE is one for induction of lens opacities; that is, low-LET particulate and low-LET electromagnetic radiations have the same cataractogenic effectiveness. The results suggested an inverse relationship between recoil-proton mean energy and RBE for lens opacities in mice.

A major investigation of the cataractogenic effects of 190 MeV deuterons and 380 MeV alpha particles on the rabbit lens was carried out by von Sallmann and co-workers (1955). The rabbits were positioned during irradiation so that the Bragg peak for both deuterons and alpha particles fell at the equatorial plane of the lens, at which point the average LET for deuterons was four keV per micron in water and 23 keV per micron in water for alpha particles. The base radiation for the RBE studies was 210 kV x-rays. The relative biological effectiveness of deuterons and alpha particles was based on degenerate cell counts in the lens epithelium for five different doses and six different times after exposure. An RBE of 4.1 was obtained for alpha particles and an RBE of 3.9 for deuterons, indicating greater cataractogenic effectiveness of the heavy ions vs 210 kV x-rays, at least for rabbits.

The effects of acute and protracted doses of protons and x-rays on the rabbit lens have been investigated by Geeraets and associates (1971).

The rabbits were exposed to x-rays, 20 MeV protons and 100 MeV protons. Four conclusions were drawn from the acute exposure data: (a) Exposure of the rabbit lens to the higher LET 20 MeV proton beam (2.57 keV/micron in water) resulted in more pronounced lens changes than exposure to the lower LET 100 MeV proton beam (0.714 keV/micron in water) at identical doses of 25, 50, 100 and 250 rad. (b) Both 20 and 100 MeV proton beams were significantly more cataractogenic than one MeV x-rays. The differential effect was more pronounced at higher dose levels. (c) Pathogenesis of cataract is identical for both protons and x-rays. (d) The latency period between irradiation and the appearance of lens changes is the same for high-energy protons and x-rays. Progression of lens changes, however, appeared slower for proton irradiation.

The fractionation studies indicated that the final degree of lens change was about equal if the total fractionated dose administered (once a month for 10 months) was twice that of a single exposure, i.e., 500 R of x-rays given over a 10 month period had the same effect as 250 R of x-rays given in a single exposure. This also held true for single and fractionated proton irradiation.

Beta Ray Cataracts

McDonald et al. (1955) have studied lens changes in rabbits due to Sr-90 beta ray applicators placed on the central cornea and on the inferior limbus. The Sr-90 sources were in equilibrium with Y-90 and had a surface output of 104 R per second. Effects noted were minimal posterior sutural opacities and vacuoles and opacities located at the lens equator.

The depth doses in the eye for this type of applicator are as

follows: 1 mm = 50%; 2 mm = 25%; 3 mm = 12%; 4 mm = 6%; 5 mm = 3%; and 6 mm = 1.3%. The equator of the lens of the rabbit is about 3 mm from the surface of the limbus and the anterior capsule of the lens is about 4 mm posterior to the center of the cornea. The minimal cataractogenic dose was found to be 500-1,000 rad applied at the surface of the limbus. Taking 12% of this minimum dose, the authors calculated that 60-120 rad reached the equatorial cells of the lens. This finding is in good agreement with the values of 75-150 R of 1.2 MeV x-rays which Cogan et al. (1953) found produced minimal changes in the rabbit lens.

The sharp localization of the beta radiation opacities and vacuoles directly beneath the area of the applicator suggested that clinical opacities caused by beta radon and Sr-90-Y-90 applicators are most likely due to direct hits or absorption of electrons on the germinative epithelial cells at the equator of the lens rather than by changes in the ciliary processes or aqueous humor, which should result in more diffuse opacities. The migration of vacuoles and opacities from the equatorial region and lens bow posteriorly to the horizontal suture can be followed clinically in rabbits who received above a minimum cataractogenic dose. These abnormal epithelial cells are found histologically beneath the capsule at the posterior pole of the lens. McDonald and his associates concluded that large doses of beta radiation produce visible changes at the lens equator first, without any evidence of posterior suture line opacities.

Work by von Sallmann et al. (1953) confirmed the fact that radiation-induced damage to the nuclei of the germinative zone is limited

to the sector closest to the irradiated part of the limbus. He noted that the area of the cornea in rabbits exposed to 2,000, 5,000, 10,000 and 20,000 rad was free of biomicroscopically visible lesions, even when cataract was present.

Radiation Effects on Lens Carbohydrate Metabolism

A number of compounds involved in carbohydrate metabolism in the lens are affected by radiation. Glutathione content in the lens is drastically reduced after radiation exposure, even before clinical detection of opacity (Pirie et al., 1953; Kinsey, 1950). One widely studied enzyme is acid phosphatase. Acid phosphatase, a lysosomal enzyme, is distributed throughout the lens epithelium but is concentrated mostly in the germinative zone where metabolic activity is greatest. Richards et al. (1969 and 1970) investigated acid phosphatase activity in the x-irradiated rabbit lens. Figure 3 shows the relative reduction in enzyme activity up to 60 days after exposure. A photoelectric meter was used to determine the relative light transmission for the central and germinative zones, acid phosphatase activity being indicated by sites of brown lead sulfide. The reduction in activity was due partly to loss of cells in the germinative zone and partly to reduction of activity within the remaining germinative cells. Since the decrease in acid phosphatase reaction in the germinative zone was not evident until approximately two weeks after exposure, it was concluded that the radiation effect on this enzyme was secondary and not direct. Indeed, in another experiment by these same investigators (Richards and Michaelis, 1971) acid phosphatase activity in the adult rabbit lens exposed to 4,000 rad of x-rays began to recover at 65 days

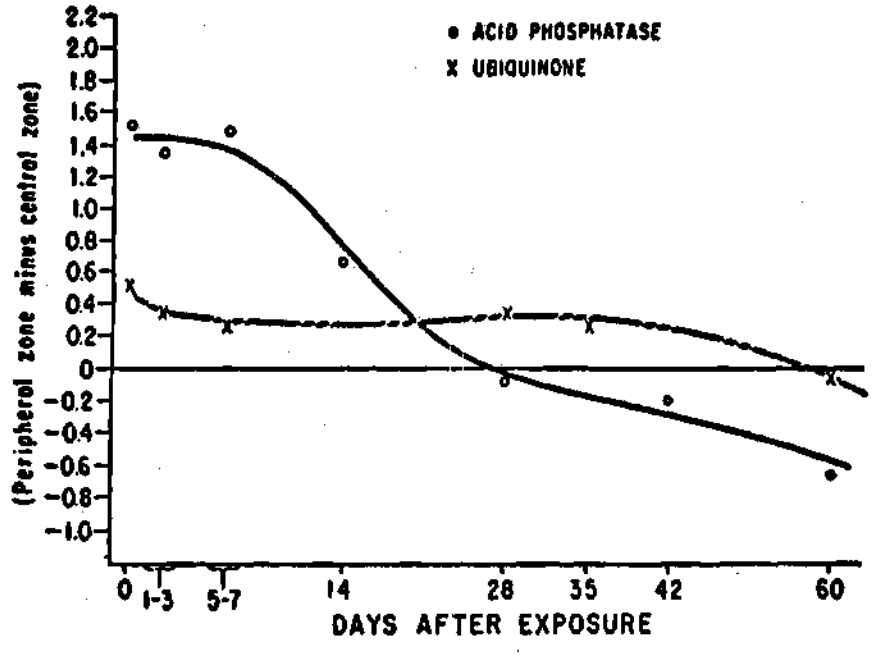


Figure 3. Decrease in Acid Phosphatase Enzyme Reaction and Ubiquinone Reaction in Rabbit Lens After Exposure to X-Rays (Richards et al., 1969)

post-irradiation when half of the lens was shielded with lead. The reason for the recovery was not apparent.

Radiation Aging of the Lens

Hockwin (1962) has demonstrated the striking similarity between radiation effects and aging in the mammalian lens. Calf lenses (under three months old) and ox lenses (over three years old) were given 100 krad in a single acute exposure (150 kV, 1,800 R/min). On the basis of lens content of ATP, ADP, and AMP, young x-irradiated calf lenses resemble closely the normally aging ox lens. He postulated that the metabolism of an irradiated young lens becomes similar to the metabolism of a non-irradiated older lens. Further, within the entire metabolic chain there may be single components with different responses to ionizing radiation. Since metabolism is a regular sequence of enzymatically controlled reactions, a disturbance of any one of these could lead to a slowing-down of or an interference with the whole chain responsible for normal growth and functioning. Exposure of the lens of adult rabbits to a single dose of 200 kVp x-rays of 1,400 R causes a deficiency in sugar metabolism at an early stage post-irradiation (Nordman and Mandel, 1955).

Radiation also seems to mimic the normal aging process in its effects on lenticular proteins. For example, it is normal during the life span of a mammal for the total amount of protein in the lens to increase with age. However, the relative fractions of soluble (in water) and insoluble protein do not remain the same. The soluble lens protein in the rat lens increases rapidly up to an age of 20 weeks when more than 90% of the maximum amount of soluble protein, reached at about one year of life,

is present (Dische et al., 1956). The soluble protein declines after the first year. The albuminoid or insoluble fraction increases during the whole life span of the animal at a slowly declining rate. Much the same thing happens after exposure of the lens to a cataractogenic dose of x-rays (Orekhovich et al., 1962; Zigman et al., 1973; Rupe et al., 1958; Philipson, 1969; Permutt and Johnson, 1953; von Sallmann, 1952). All evidence points to a decrease in the soluble fraction with a concomitant increase in the insoluble fraction; in fact, there is a good indication that radiation actually accelerates the conversion of soluble protein into insoluble (albuminoid) protein. This transformation has been demonstrated after exposure of the lens to near ultraviolet light (Zigman et al., 1973).

Protection from Radiation Cataract by Partial Lens Shielding

Radiation damage to the lens can be lessened by partially shielding one or more of the lens quadrants during irradiation (Pirie and Drance, 1959). This lessening of damage, which is evident by a less severe cataract, fewer fragments of cell nuclei, smaller mitotic overshoot and reduced cell loss in the irradiated epithelium, does not appear to be due to cell migration from healthy to irradiated areas since the area of damage does not decrease in size with time. It is possible that damage is minimized due to diffusion of substance into the irradiated area from other parts of the lens. When part of the lens is shielded, part of the ciliary body also escapes irradiation and protective substances may diffuse from the aqueous humor into the damaged epithelium.

Radiation exposure damages both the lens fibers and the epithelial

cells. Whatever mechanisms are at work in the recovery of half-irradiated lenses, these mechanisms must effect both types of damage. The protective effect of partial shielding was observed in rats irradiated acutely with 250 kVp x-rays with one half of the lens shielded by a lead corneal contact lens (Fenske and Leinfelder, 1966). The partial shielding prevented formation of a complete cataract. The lens epithelium seemed to recover with the production of normal lens fibers. There were fewer pathological lens fibers formed and fewer cataractous changes.

Shielding of a part of the lens improves recovery after irradiation. Complete cataracts developed when an entire rabbit lens was exposed to 2,500-11,000 rad in a single exposure (Riley et al., 1958). The radiation damage did not progress to complete opacification, even after exposures of 11,000 rad, if at least one quarter of the peripheral epithelium was shielded during exposure. One half of a rabbit lens was exposed also to 11,000 rad and then, after various intervals of time, the second half was also exposed to 11,000 rad. When the interval between exposures was three months or less, a complete cataract always ensued. When the intervening time was at least four to five months the opacification was limited in extent. This indicated that the half which was irradiated first was able to recover enough in four to five months to sustain the lens and enable the second half to recover when it was in turn irradiated.

No opacity at all is produced when only the axial core of the rabbit lens is irradiated acutely with 1,600 rad of 250 kVp x-rays (Pirie and Flanders, 1957). An acute dose of 1,400 rad given to the whole eye, however, causes the development of complete cataract in 5-10 months. A

dose of 1,600 rad delivered in a single, brief exposure to the lens periphery produces cortical opacities within that area. It has been found that a complete cataract develops only if more than three fourths of the lens periphery is irradiated.

Richards and associates (1956) observed lens changes in rabbits following x-irradiation of single and multiple lens quadrants. The opacities in lenses exposed in all portions to 3,000 R or more of 250 kVp x-rays progressed rapidly to maturity. In contrast, opacities in partially shielded lenses stopped progressing and eventually appeared to regress. After massive doses to only a portion of the lens, it was again able to produce normal fibers at some time after irradiation. With larger doses to the entire lens, the formation of pathologic fibers continued and eventually fibers formed prior to irradiation began to degenerate. Shielding of a portion of the lens was found to prevent the progression to complete cataract, even after exposures of 12,000 R.

Some investigators believe that damage to the ciliary body plays an important role in radiation cataractogenesis (Puntenney and Shoch, 1954). The consensus, however, is that injury to this tissue is only a minor factor in the pathogenesis of cataract. Irradiation of the ciliary body alone has never been observed to cause cataract (Shoch and Puntenney, 1951; Alter and Leinfelder, 1953).

Radiation Cataract and Species Sensitivity

The lenses of all mammalian species respond qualitatively in the same manner to ionizing radiation (von Sallmann, 1957). There is great variation, however, in quantitative response, measured both clinically

and histologically. Von Sallmann (1957) observed that the onset of opacities was delayed in dogs and monkeys as compared to rats, guinea pigs, cats, mice and rabbits. The lenses of adult monkeys and dogs have the smallest number of dividing cells (calculated per 100,000 cells) among the species studied. This low mitotic activity may be correlated with the relatively lower radiosensitivity of these lenses.

There is a systematic change according to body size in the sensitivity of the lens to neutron damage, smaller animals being more sensitive than larger animals (Edmondson et al., 1967). Goats which survived single, whole-body neutron exposures of 100-633 rad were examined at intervals up to three years after exposure. The LD 50/30 for goats for single bilateral whole-body neutron exposure was 505 rad. Only two out of 28 goats receiving fission neutron radiation showed changes in the lens and this occurred at the highest dose given of 633 rad. No radiation-induced changes in the lens were found after whole-body gamma ray doses of 200-600 R. Table 5 gives a comprehensive summary of primate sensitivity to radiation cataract.

Furth et al. (1954) observed cataracts among the late effects seen in 6-12 week old mice exposed to radiation from an experimental nuclear detonation. The radiation was predominantly high-energy gamma rays with a small component of fast and slow neutrons. The opacities appeared during the third month post-exposure. Within 90 days after exposure almost all irradiated mice had opacities detectable with the slit-lamp; their rate of progression varied directly with dose. The mild opacities induced by small doses, up to 200 rad, did not progress to form marked cataracts

Table 5. Summary of Species Variation in Sensitivity to Radiation Cataractogenesis

Species	Radiation	Lethal dose for single whole-body exposure (usually LD 50/30)	Single exposure cataractogenic dose (WB = whole body, L = lens)		Neutron/gamma RBE	
			Marked opacification 1-2 years	Lens changes detectable but not likely to interfere with vision	Lethality	Marked opacification
Swiss mice	X-rays 250 kvp Fission neutrons	575 R 210 rep	775 R (WB) < 175 rep (WB)	100 R (WB)	2-4	'circa 5'
RF mice	X-rays 250 kvp Fast neutrons 1-2 MeV Fast neutrons 2-3 MeV	530 R 360 rep	> mid-lethal (WB) 280 rep (WB)	33 R (L) < 160 rep (WB) 1-3 rep (L)	1-5	3
Rats	X-rays 250 kvp Fast neutrons 1-2 MeV Fast neutrons 1-2 MeV		640 R (WB) 360 rep (WB)	< 240 R (WB) < 180 rep (WB) 12-37 rep (WB)		2-3
Guinea-pigs	X-rays Fast neutrons X-rays 250 kvp Fast neutrons 1-2 MeV (mean)	275 R 155 rads	 > 360 R (WB) > 360 rep (WB)	 120 rep (WB) 90 rep (WB)	1-8	 2-3
Rabbit	Gamma 2-5 MeV (mean Fission neutrons 0-7 MeV (mean) X-rays 1-2 MeV Fission neutrons	1389 R 572 rads	 465 R (L) 54 rads (L)	 250 R (L) 2-7 rad (L)	 2-4	 9
Dog	X-rays 250 kvp Fast neutrons X-rays 1000 kvp X-rays Fast neutrons 6-8 MeV	252 rep 289 rep 280 R	 1215 R (upper body)	 > 300 R (WB) 150 N (375 rads)(L)	0-8	
Monkey	^{60}Co gamma ^{60}Co gamma Fast neutrons 1-4 MeV Fission neutrons Fission neutrons	438 R 403 rads	 > 250 rep (L)	 500 rep (L) 75 rep (L) > 150 rads (WB)	 1-20	 4
Goat	Gamma 2-5 MeV (mean) Fission neutrons 0-7 MeV (mean)	395 R 505 rads	 > mid-lethal (WB)	466 rads (WB)	 0-8	
Man	X or gamma X-rays Gamma (radon seeds)		 > 1000 R (L) > 2000 R (L) (low dose rate)	200 R (L) > 400 R (L)		

NOTE: Taken from ICRP #14, 1969.

which would interfere with vision even after 22 months post-irradiation, whereas opacities caused by larger doses progressed to complete cataracts, the rate of progression depending on the dose. Mice surviving an exposure of 850 rad developed complete cataracts within one year after the detonation.

Oxygen Enhancement of the Radiosensitivity of the Mammalian Lens

The presence of dissolved oxygen during irradiation is known to increase the effectiveness of x-rays in causing injury or death to cells from many different organisms (Howard-Flanders and Pirie, 1957). Observations on the treatment of human malignant disease by radiotherapy have shown that portions of a tumor which are poorly supplied with oxygen are resistant to radiation. Groups of malignant cells may survive an irradiation which would be lethal to well-oxygenated cells. The sensitivity of cells in parts of the tumor which were previously anoxic may be increased differentially, with respect to the surrounding normal, well-vascularized tissue, by administering oxygen at a higher partial pressure.

Well-vascularized tissues such as skin appear to be at almost their maximum radiation sensitivity when the patient is breathing air. Therefore, the administration of oxygen may lead to a greater increase in the sensitivity of poorly vascularized tissue than occurs in those with a good oxygen supply, such as skin. The lens is poorly oxygenated, being supplied with oxygen at the surface only, and one would expect an increase in lens oxygen content to enhance its radiosensitivity. Lens oxygen is supplied through the lens capsule from the aqueous humor. The oxygen tension in the aqueous humor of an animal breathing air is about 54 torr;

with 95% oxygen and 5% CO_2 it rises to 230 torr. At this pressure the oxygen tension in the germinative zone of the lens epithelium is around 20 torr.

Howard-Flanders and Pirie (1957) irradiated rabbits with 109 kV x-rays with the animals in a Lucite dome, breathing either air or oxygen at one atm pressure. The distance from the center of the eye to the x-ray target was 22 cm and the dose rate was 130 rad/min. Doses were measured with a Baldwin-Farmer substandard dosimeter placed at the same position of the eye and surrounded with material to exactly reproduce the conditions of absorption and scattering due to the animal.

The mean value for the ratio of the doses in air and in oxygen (95% oxygen + 5% CO_2) required to produce the same percentage fall in lens glutathione was 1.24. It was found that rabbits breathing oxygen at one atm pressure were 24% more radiosensitive to reduction in lens glutathione than were animals breathing normal air.

Radiation Cataract in Man

Our experience with ionizing radiation cataract in man comes primarily from three sources: surveys of the survivors of the atomic bombing of Hiroshima and Nagasaki, Japan; cyclotron workers whose eyes were exposed to beams of fast neutrons and hard gamma rays; and patients who received radiation therapy about the region of the orbit and later developed lens opacities. In addition there is scattered information regarding isolated cases of occupational exposure to both ionizing and non-ionizing radiation in industry.

The first investigators to indicate that x-radiation can give rise

to cataracts in man were Gutman and Treutler, who reported cases in radiological technicians in 1905 (Duke-Elder and MacFaul, 1972).

Perhaps one of the earliest known cases of cataract in man following exposure to high LET radiation involved an individual exposed in 1945 to the mixed radiation emitted when an experimental assembly of fissionable material became critical and who later developed cataracts (case #4 of Hempelmann, Lisco and Hoffman, 1952). Epilation of the left side of the scalp occurred during the third and fourth weeks after exposure. At 28 months post-exposure both lenses were normal but by 38 months post-exposure there was a moderately advanced radiation cataract of the left eye. Opacities of the right eye were seen for the first time about five years after exposure.

Cyclotron Physicists

Early in December 1948 it became known that several physicists in the United States who had been working with cyclotrons had developed radiation cataracts (Abelson and Kruger, 1949). It was concluded that exposure to fast neutrons was the probable cause of the cataracts. Two affected individuals showed mild epilation of the head which indicated that their exposures were greatly in excess of anything that might have been considered safe. The doses cited by Abelson and Kruger (1949) for their three cases with definitely impaired vision were 160, 160 and 270 rad. Dollfus (1950) recorded four cases of opacification of the lens in cyclotron workers in France. The only subject with impaired vision, a left-handed man, showed epilation of the left forearm.

Soon after the discovery of cataracts in physicists exposed to cyclotron radiation, an ad hoc committee (the Committee on Radiation

Cataracts) was established by the Division of Medical Sciences of the National Research Council. This committee sent questionnaires to all of the high-voltage laboratories across the United States where comparable exposure risks might have been incurred, requesting that these physicists come to Washington.

A meeting was held in Washington, D.C. on January 16 and 17, 1949, under the auspices of the Division of Medical Sciences of the National Research Council with the support of the Atomic Energy Commission. On January 18, 1949, 11 of these exposed physicists were examined by slit-lamp, 10 of whom showed opacities in the lens. They were divided into three groups: three men showed severe changes in the posterior cortex of the lens and complained of reduced vision. Four had slight to moderate changes in the posterior cortex. Three individuals showed only minimal and questionable changes. The severe cases did not fully develop until about three years after the radiation was received. These workers were receiving periodic blood examinations at the time of the exposures but no hematologic effects were seen.

In February, 1950, the Committee on Radiation Cataracts held a meeting in Washington to assess the status of the problem and to plan a definitive research program. One objective of the committee was to stimulate research in radiation dosimetry. Since that time there have been at least six major conferences on radiation cataracts.

Atom Bomb Survivors

A number of surveys have been conducted on the survivors of the atomic bombings of Hiroshima and Nagasaki, Japan (Fillmore, 1952; Cogan

et al., 1949; Sugimoto, 1962; Sinskey, 1955). Radiation cataracts were seen in 98 survivors at Hiroshima at about five years after exposure. Seventy-seven of them had also suffered epilation of the scalp. Miller and associates (1967) have summarized lens findings in atomic bomb survivors in a review of major ophthalmic surveys conducted by the Atomic Bomb Casualty Commission from 1949 to 1962. Table 6 shows the incidence of posterior opacities with distance from the hypocenter at which exposure occurred at Hiroshima and Nagasaki. Masuda (1967) published a survey dealing with observations on 1101 persons exposed to atomic radiation at Hiroshima. This survey was conducted eight to thirteen years after exposure. His main findings were: (1) Vision was more favorable than expected. (2) The overall incidence of lens opacities was 9.5% in 231 unexposed individuals and 19.7% in 1101 exposed individuals. The highest incidence of lens opacities attributed to radiation was 19.6% in 280 individuals with epilation. However, about half the attributable opacities occurred in individuals without epilation.

Over 2500 survivors have been examined and followed through 1964. Estimates in 1965 of the air dose received by survivors ranges from 450 rad at 1000 meters from the Hiroshima hypocenter to about two rad at 2000 meters, and from 925 rad to about 18 rad in Nagasaki at these distances. The log of the air dose in rad was determined to be linearly related to distance from the hypocenter.

All surveys conducted by the Atomic Bomb Casualty Commission reported changes in the axial posterior subcapsular region of the lenses of exposed persons, which became more severe the closer the exposure occurred

Table 6. Axial Posterior Opacities by Distance from the Hypocenter at Hiroshima and Nagasaki (1949-1950)

Distance Meters	No. Examined	Axial Opacities	
		Number	% Incidence
less than 600	68	9	13.2
600-799	249	23	9.2
800-999	550	52	9.5
1000-1199	908	16	1.8
1200-1399	223	0	0.0
1400-1599	209	0	0.0
1600-1999	712	0	0.0
2000 +	66	0	0.0

NOTE: Taken from Miller et al., 1967.

to the hypocenter. The radiation cataracts were divided into three categories:

1. Severe lens changes--complete opacities, posterior subcapsular plaque, doughnut opacity, with resultant diminution of central visual acuity

2. Moderate effects of irradiation--small irridescent posterior subcapsular granular opacity; no significant reduction in vision

3. Minimal radiation effects--consisting of a polychromatic sheen in the plane of the posterior capsule of the lens and apparent only with slit-lamp

Of all the patients examined at Hiroshima and Nagasaki only 20 were in the first category. Among the small group of survivors within 500 meters of the hypocenter, 15% exhibited moderate changes. It should be remembered that these slight changes are seen in people of any age with no history of exposure to ionizing radiation.

Conclusions Regarding the Effects of Fission Neutrons and Gamma Rays on the Human Lens

The following conclusions about the effects of fission neutrons on the human lens were drawn based on the evidence of the surveys of atom bomb survivors: (1) Cataract of a degree sufficient to impair vision develops within a few years following a single large exposure to fission neutrons and gamma rays and the necessary dose is almost invariably sufficient to epilate the scalp. (2) Lens opacities of a lesser degree detectable by slit-lamp examination but without effect on vision may follow a sub-epilatory dose. (3) Most lens opacities which do not interfere with vision do not progress; they may regress with time even when the cataractogenic agent is high LET radiation. (4) There is a sigmoid dose-response

for high LET radiation for the production of cataract of a degree sufficient to impair vision, i.e., the human lens shows a threshold response in that a minimum radiation dose is required to produce a defined degree of change. (5) For fast neutrons a cumulative dose of 75-100 rads approaches the threshold for cataract in man. This is considered to correspond to a maximum permissible dose to the lens of 15 rem per year of fast neutron radiation for chronic exposure (ICRP #14, 1969).

Cataracts Induced by Radiation Therapy

The first case of radiation cataract after therapeutic radiation was published by Sir Arthur Birch-Hirschfeld in 1908 (Duke-Elder and MacFaul, 1972). By 1932, 50 cases of x-ray cataract had been reported. Neutrons were used in radiotherapy of patients from 1938 to 1943 but no cases of neutron cataract were reported in the literature during that period (Krause and Bond, 1951).

Hunt (1947) surveyed 100 patients selected from 134 cases of eyelid cancer treated by x-radiation in the years 1930-1946. He concluded that the threshold dose of low LET radiation for cataract induction in the human lens is about 520 rad in tissue. Cataracts had been observed clinically about five years after an x-ray exposure of 775 rad to the lens and seven years after an exposure of 1000 rad. Vishevskii et al. (1961) however have placed the minimal x-rays dose for cataract formation in man at about 200 R, with a latent period of two to seven years.

A number of cases of cataract in man have been reported following treatment of cancer by Sr-90 beta ray applicators and by radon gold seed implants (Cogan and Dreisler, 1953; Leinfelder, 1936; Hilgers, 1966;

McDonald et al., 1955; Merriam and Focht, 1957; and Britten et al., 1966). The threshold dose for beta ray cataract due to radon gold seed implantation in the eyelid has been determined to be about 2000 R, with doses of 4000 R or more always resulting in cataracts.

Conclusions Regarding the Effects of Low LET Radiation on the Human Lens

The dose-response for cataract induction by acute exposure to low LET radiation is highly sigmoid, with an apparent threshold dose below which no lens opacities are seen (ICRP #14, 1969). The minimum dose of low LET radiation for just detectable lens opacification is larger the more protracted the dose. Also there is an inverse relationship between radiation dose and the latency period for initial lens changes.

Table 7 summarizes the dose-response relationship for cataract induction in man following exposure to low LET radiation. It seems to be true in general that minor lens opacities induced by both low and high LET radiation which can be detected by skilled examination and which do not interfere with vision are usually non-progressive and may even improve in the post-exposure period.

Merriam and Focht (1957) suggest that the dose limit for a lifetime of occupational exposure of the lens should be 15 R per year of low LET radiation. After 50 years the accumulated dose would be 750 R, whereas their data indicated that 550 R was the threshold dose for producing detectable lens opacities when the exposure was protracted over three months to eight years. In allowing personnel to receive an additional 200 R above the apparent threshold for lens opacity the authors assumed that

Table 7. Lens Opacities and Dose of Low LET Radiation in Man

Authors	Radiation	Overall duration of exposure	Minimum dose at which lens change was at detected R	Grade of change "minimum" dose	No. of individuals with lens opacities/ No. of individuals examined					
					Dose range in fractions of minimum dose for detectable opacification					
					0-	$\frac{1}{2}$-	1-	$1\frac{1}{2}$-	2-	
(10 years +)										
Cogan and Dreisler (1953)	X	I "Single" exposure	600	Just visible by use of opthalmoscope or slit lamp			1/3		1/1	
		II 3 weeks-3 months	800		0/20		2/2		1/1	
Merriam and Focht (1957)	Mostly radium	I Single exposure	200		0/9	0/8	2/2	2/2	16/16	
		II 3 weeks-3 months	400		0/10	0/9	4/4	2/3	25/26	
		III Greater than 3 months	550		0/1	0/5	2/4	0/1	15/15	
Britten et al. (1966)	Radon gold seed		1600	Minor (not noticed by patient)		0/8	2/12	4/7	7/11	Same subjects classified in two different ways
			3100	Major (vision impaired)		0/18	4/10	1/2		
INFANTS AND CHILDREN										
Merriam and Focht (1957)	Mostly	II 3 weeks-3 months	560	Just visible by use of opthalmoscope or slit lamp			4/7	13/23	1/1	
		III Greater than 3 months	550		0/2	0/1	2/3	1/2	11/12	
Qvist and Zachau-Christiansen (1959)	Radium		1380		0/52		2/2		2/2	

NOTE: Taken from ICRP #14, 1969.

protracting the exposure over a period of 50 years would be sufficient to offset this increase in allowable dose. Whether this assumption is really justified is unknown.

The evidence from radiation therapy points to the fact that there appears to be an apparent threshold dose (about 200 R of low LET radiation) below which we do not find lens opacities. When the total dose to the lens exceeds 1,100-1,400 rad, there is a high probability of cataract formation, irrespective of the duration of exposure.

No information is available about the range of sensitivities of human eyes to ionizing radiation (ICRP #14, 1969). Experimental work is still needed to see if senile cataract in man is augmented or accelerated by exposure to radiation, and a synergistic interaction of radiation and age still remains a possibility.

Ion Levels in Normal and Cataractous Lenses of Man and Other Vertebrates

It has long been appreciated that lens cataracts are often associated with very marked changes in normal ion levels. Burge (1909) was probably the first to observe that human cataractous lenses contained much more sodium and calcium and much less potassium than did normal lenses. These findings were confirmed by Adams (1929) and by MacKay, Stewart and Robertson (1932) who also reported that the chloride concentration was markedly increased. Adams (1929) was the first to investigate in detail the role of calcium in lens cataracts. She confirmed the great increase in calcium in cataractous lenses, a trend which is highly significant since the normal lens has one of the lowest calcium contents of any tissue (Kuck,

1970).

Duncan and Bushell (1975) investigated the concentrations of sodium, potassium, calcium, magnesium and chloride in 52 human cataractous lenses. A group of lenses having mixed cortical and nuclear cataracts possessed significantly raised sodium and lowered potassium concentrations but particularly a raised calcium concentration. Lenses with mainly cortical and mature cataracts had greatly increased sodium and calcium concentrations, increased chloride concentrations and very low potassium levels. The magnesium concentrations remained relatively constant in both normal and cataractous lenses. Maraini and Mangili (1973) demonstrated that the sodium and potassium content of lenses with pure nuclear cataracts were not significantly different from normal lenses. Lenses with cortical cataracts, however, had markedly raised sodium levels and lowered potassium levels. These findings raise the possibility of classifying cataract type on the basis of ionic composition.

Similar trends in ion levels have been observed in lenses with cataracts induced by x-irradiation. Chubakov and Luzanova (1960) analyzed the ionic levels of the cataractous lens of the rabbit using thermal neutron activation analysis. Rabbit lenses were first exposed *in vivo* to 1,000 R of 200 kVp x-rays to produce cataracts. One year after x-ray exposure the lenses were enucleated and then irradiated in a thermal neutron flux of 10^{14} n/cm^2-sec for a period of 30 minutes. Normal and x-irradiated lenses were activated under identical conditions and the induced beta ray activity then counted on a front window detector. The cataractous lenses were found to be almost totally depleted of potassium while

the sodium levels had risen to ten times normal levels. The chloride content had increased by a factor of four in exposed lenses. No variation in phosphorus was detected.

Changes in ion concentrations are much less dramatic in the normally aging lens (Kuck, 1970). The potassium concentration in the whole lens is not altered in aging; a slight but significant rise in sodium concentration leads to a decrease in the K/Na ratio in the mammalian lens. This trend is indicative of a tissue mass which is becoming less viable and less able to extrude sodium and prevent a net loss of potassium. The levels of sodium, potassium and calcium in the rat lens have been shown to decrease asymptotically with lens age when the values are expressed as microequivalents of ion per mg lens wet weight. The relatively greater accumulation of calcium in cataract is well substantiated, so if the parallel trends for these three cations found in the rat lens during normal aging can be shown to be generally true for all lenses, then the determination of calcium levels should be a valuable tool for contrasting the normal aging lens and that developing cataractous changes.

The investigation of ionic levels in normal and cataractous lenses raises a question over what is a rational basis for expressing the concentrations of lenticular ions. For example, the curve for percent sodium in aging lenses as a function of percent water in the lens has constant slope if the sodium levels are calculated on a lens wet weight basis but has an appreciable positive slope if these levels are expressed on a dry weight basis instead. It is suggested that ionic concentrations expressed as millimoles or milligrams of ion per unit of lens wet weight may give a less misleading picture of lens behavior than concentrations based on dry

weight or lens water. This argument applies especially to sodium and potassium in the normal lens (Kuck, 1970).

Significant changes in the concentrations of trace metals are also observed in normal aging lenses and lenses undergoing pathological changes (Kuck, 1970). Zinc and titanium accumulate with age in the lenses of man and several vertebrates, while copper, cobalt and nickel decrease with age. Normal human lenses are high in zinc whereas lenses with senile cataract lose as much as 75% of their normal zinc content. Senile cataracts have also been found to be high in cobalt, iron, and copper. The reduction in zinc and elevation of copper in human cataract is interesting in that exactly opposite trends occur for these elements in blood. Representative values for selected trace elements in the adult ox lens are presented in Table 8.

Summary of Current Knowledge of Radiation Cataractogenesis

In the light of current knowledge about mammalian lens response to ionizing radiation, the following conclusions seem justified:

(1) The mammalian lens is highly sensitive to ionizing radiation and this sensitivity manifests itself as a late radiation effect in the form of opacities which develop within the lens following *in vivo* exposure to radiation.

(2) There exists a latency period between radiation exposure and the clinical appearance of opacity which is an indirect function of radiation dose.

(3) A dose of 200-600 rad of low LET radiation appears to be required to cause detectable changes within 10-15 years in the human lens

Table 8. Concentrations of Selected Trace Elements in the Normal Adult Ox Lens

Element	Concentration (mg element/ 100 g lens wet weight)
Zinc	1.5
Aluminum	0.5-5.6
Silicon	0.8
Iron	0.6
Manganese	0.012-0.13
Tin	0.2
Lead	0.04
Molybdenum	0.03
Copper	0.046
Silver	0.006-0.06
Strontium	0.006
Barium	0.005

NOTE: Taken from Kuck, 1970.

when the dose is given in a single brief exposure.

(4) The evidence from radiation therapy points to the fact that there appears to be an apparent threshold dose (about 200 R) of low LET radiation below which we do not find lens opacities, up to 20 years after exposure.

(5) Radiation-induced lens opacities may progress, remain stationary, or even regress. As the dose increases the probability of progression of opacity increases.

(6) Fractionation or protraction of radiation exposure increases the threshold dose for cataractogenesis, especially for low LET radiation. If the low LET dose is given over several months, instead of during a brief single period, two to three times as much radiation may be required before cataracts are observed. However, when the total dose to the human lens exceeds 1,100-1,400 rad, there is a high probability of cataract formation, irrespective of the duration of exposure.

(7) Fragmentary data on the effects of high LET radiation on the human lens are consistent with a threshold cataractogenic dose of 70-100 rad of fast neutrons. The RBE for cataractogenesis induced by fast neutrons is greater than unity and fractionation does not appreciably reduce the effectiveness of high LET radiation.

(8) The dose-response for cataract induction by ionizing radiation, whether of high or low LET, seems to be highly sigmoid.

(9) Lens cataracts of all types are often associated with significant changes in ion concentrations.

CHAPTER V

OBJECTIVES OF THIS RESEARCH AND SELECTED PROCEDURE

As discussed in the previous chapter on the current knowledge regarding ionizing radiation cataracts in the mammalian lens, the lens is an organ that is especially sensitive to radiation damage and any radiation injuries which it sustains are manifested as opacities that are maintained in perpetuity. Lens cataracts of all types, including ionizing radiation cataracts, are often associated with significant changes in ion concentrations.

Of special interest to lens researchers is the detection of such ion changes well in advance of the clinical appearance of opacity. Since the specific mechanisms by which cataracts are induced in the lens by ionizing radiation are still unknown, the demonstration of chemical changes prior to cataract development could help to clarify the etiology of radiation cataract.

Based on the previous observations of the relationship between lens cataracts and ion levels, the following objectives were chosen for the present research:

(1) The detection and measurement of changes in ion concentrations in the lens after exposure to ionizing radiation but prior to the clinical appearance of cataract.

(2) The investigation of the variation in specific ion levels in the lens with radiation dose and time post-exposure.

(3) The correlation of clinical examination of the cataractous lens by slit-lamp examination with quantitative analysis of ion changes in the lens.

(4) The determination of the variation of ionic concentrations in the lens with normal lens aging.

(5) The testing of the radiation dose-response threshold hypothesis on the basis of changes in ion levels in the lens following ionizing radiation exposure.

An analysis of the chemical composition of the cataractous lens might be one method of classification of cataract type. In view of the work of Maraini and Mangili (1973), which demonstrated markedly different ionic levels in lenses with cortical cataracts relative to those with pure nuclear cataracts, cataracts such as senile, radiational, diabetic, etc. might be differentiated by differences in ionic composition. Pirie (1968), van Heyningen (1972), and Maraini and Mangili (1973) have emphasized the need for an objective criterion of cataract type.

The most common clinical methods of assessing radiation damage to the lens have been slit-lamp examination and histological techniques. Slit-lamp biomicroscopy by nature is subjective and histological techniques usually do not yield precision measurements. The sensitivity of the slit-lamp examination is partly a function of the expertise of the investigator using it, but in general it can indicate pathological changes in the lens many months to years before these changes begin to noticeably interfere with vision.

After reviewing these methods, it was decided to investigate both

normal and cataractous lenses of experimental animals for ion content and cataract development using both thermal neutron activation analysis and slit-lamp biomicroscopy. Thus, this method of approach to the problem combines the extreme sensitivity of activation analysis (in some cases down to parts per billion) for some of the important elements in the lens with visual clinical assessment of the lens state at any given time after irradiation.

Activation analysis can be much simpler than multi-element trace determinations using ordinary wet chemical procedures and often no complex chemical separations are required. By optimizing sample activation time and the delay period between activation and counting, often as many as twenty different elements can be identified and measured by this method. Also, activation analysis is usually non-destructive. The limitations of this method generally depend on the percentage abundance of the unknown or parent nuclide, its concentration in the sample, its thermal neutron cross section, the thermal neutron flux at the irradiation position in the reactor, and the decay scheme of the resulting activation product (e.g., the absolute gamma ray intensities).

Table 9 summarizes the neutron activation parameters of some of the important naturally occurring elements in the normal mammalian lens. It is these factors, combined with the normal concentration of the elements in the lens, which ultimately determine the sensitivity of neutron activation analysis for specific elements. For example, calcium normally occurs in small amounts in the lens, but Ca-49 was not detected in this project due to low detector efficiency for its principal gamma ray, its

Table 9. Neutron Activation Parameters of Naturally Occurring Elements in the Mammalian Lens Listed in Order of Increasing Atomic Number

Principal Activation Product	Principal Gamma Ray Peak (keV)	Thermal Activation Cross Section of Parent (barns)	Percent Abundance of Parent Nuclide	Half-life
Na-24	1369 (100%)*	0.53	100	15.0 hr
Mg-27	840 (70%)	0.03	11.3	9.5 min
Al-28	1780 (100%)	0.24	100	2.3 min
P-32	pure β	0.19	100	14.2 d
S-35	pure β	0.27	4.2	87.9 d
Cl-38	1642 (38%)	0.40	24.5	37 min
K-42	1525 (18%)	1.2	6.77	12.4 hr
Ca-45	pure β	0.7	2.1	165 d
Ca-49	3100 (89%)	1.1	0.185	8.9 min
Mn-56	848 (99%)	13.3	100	2.6 hr
Cu-64	pure β	4.5	69.1	12.8 hr
Zn-65	1116 (49%)	0.46	48.9	245 d

*The percentages given in the second column refer to the absolute intensities of the principal gamma ray peaks.

NOTE: Taken from Radiological Health Handbook, 1970.

short half-life (8.8 min.), and the low percentage abundance of the parent nuclide (0.185). Phosphorus, sulfur, and copper were not detected in the lens by gamma ray spectroscopy due to the fact that their principal activation products P-32, S-35, and Cu-64, respectively, are pure beta emitters. Magnesium and aluminum were not observed primarily due to their very short half-lives.

Two different animals were chosen for this project: inbred black mice, strain C57BL, and Mongolian gerbils. Sprague-Dawley inbred mice were chosen for a number of reasons: they are prolific breeders (gestation period of only 19 days), they usually have relatively low mortality rates, their inbreeding minimizes individual variations, they are readily available and they are known to develop radiation cataracts. Also, their lenses are relatively large for an animal of their size. Mongolian gerbils were chosen because very little work has been done concerning radiation cataractogenesis in this species and their pigmented iris enhances slit-lamp examination of the lens. The small body of literature describing research using M. unguiculatus indicated that it possesses great potential for a wide variety of research purposes and is deserving of investigation in its own right. The study of radiation effects on gerbils seemed worthwhile in view of the investigations of Chang, Hunt, and Turbyfill (1964), in which it was found that the Mongolian gerbil's resistance to gamma ray irradiation from a radiocobalt source is about twice that of the commonly used experimental mammals, as measured by the LD 50. In particular, it was desired to determine if the gerbil lens also exhibits this radioresistance when compared to lenses of

common laboratory animals.

Burns (1956) demonstrated that G. gerbillus possesses a great capacity for temperature regulation and a high degree of tolerance for heat, features which contrast with those of common laboratory animals such as mice, rats, and rabbits. Also, Billingham and Silvers (1963) have indicated that M. unguiculatus possesses only a few histocompatibility genes, indicating potential value in studies of cancer, aging, tissue transplantation, and immunogenetics.

Although gerbils are not as widely used in laboratory experiments as mice and rats, they are available at costs comparable to that of these animals. Gerbils mate for life and are docile and easy to handle in most cases.

Based on previous experiments with mice reported in the literature, radiation cataracts are expected to appear three to four months after a single acute exposure of 800-1000 rad of x-rays to the lens if the animals are four to eight weeks old at the time of exposure. Older mice (e.g., five to six months of age at the time of exposure) would not be expected to develop cataracts at this dose level until about ten months post-exposure. No information regarding radiation cataract in gerbils was found in the literature.

In view of the above latency periods and the time constraints imposed on this project, it was decided to investigate the cataractogenic effects of x-rays on the mouse lens in the dose range from 360-1500 rad at two different age groups: 4-8 weeks and 20-24 weeks. In addition to the x-ray exposures it was also decided to investigate the cataractogenic

effects of thermal neutrons and fast neutrons on the mouse lens. Mongolian gerbils played only a minor role in this thesis and these animals were given only a single acute dose of 1000 R of 160 kVp x-rays.

The following radiation sources were found to be available for this investigation: a Picker Orthovoltage x-ray unit capable of operating safely up to about 180 kVp; the Bio-Medical facility of the Neely Nuclear Research Center at Georgia Tech, which was designed for radiotherapy of human cancer patients and provides a mixed radiation beam of thermal neutrons and gamma rays with a very small fast neutron component; and the Health Physics Research Reactor at Oak Ridge National Laboratory, a source of fast neutrons with relatively low gamma ray contamination. In addition, a number of other x-ray and neutron sources were available at Georgia Tech, but their dose rates were not in a range suitable for cataract work of the present kind.

CHAPTER VI

EXPERIMENTAL PROCEDURE

Animals

Fifteen C57BL inbred black mice (five males and ten females) were obtained for breeding from the Sprague-Dawley Co., Madison, Wisconsin. In addition, 31 Mongolian gerbils (Meriones unguiculatus), twelve months of age, were obtained from the Cappet Corporation, Lithonia, Georgia. The original 15 mice, four weeks old at the time of shipment, were used exclusively for breeding purposes; the first generation offspring of these breeders were used as the controls and irradiated subjects in this project. Sixteen gerbils served as controls (eight females and eight males) and 15 (eight males and seven females) were irradiated with 160 kVp x-rays. The gerbils were maintained in pairs until irradiation, after which they were kept one to a cage. Breeding of berbils was not successful and was abandoned early in the project.

All animals were housed in stainless steel cages under standard laboratory conditions. Food and water were provided ad libitum and lighting was controlled automatically by a timer. The gerbils were maintained on Eight-In-One^{R} Gerbil Food and cedarwood bedding; in addition they were dusted weekly for mites with 5% Sevin^{R} dust. Mice were kept on Purina Mouse Chow^{R} and San-i-cel^{R} laboratory bedding (ground corn cobs). Tap water was provided for both mice and gerbils, although gerbils require almost no water as such (Arrington and Ammerman, 1969).

Fifteen gerbils were selected at random and given a single, acute dose of 1,000 R of 160 kVp x-rays. The whole-body, with the exception of the head, was shielded with 3/4 inch of lead. The gerbils were anesthetized during irradiation by intraperitoneal injection of Nembutal^{R} (sodium pentobarbital), 47.6 mg per kg body weight, to maintain a fixed exposure geometry. The sixteen control gerbils were sham irradiated and also anesthetized with Nembutal^{R}.

The C57BL inbred mice were given single, acute doses of either 160 kVp x-rays, thermal neutrons, or fast neutrons. As with gerbils, the mice were given whole-body shielding. With the exception of 15 mice which were exposed to fast neutrons, all irradiated mice were also anesthetized with Nembutal^{R} during exposure. Control mice were also sham irradiated and given anesthesia. The schedule of exposures of mice to x-rays, thermal, and fast neutrons is presented in Table 10.

Radiation Sources

All x-ray exposures were carried out at 160 kVp using a Picker Ortho-voltage unit capable of operating safely up to about 180 kVp. The x-ray exposures were given under the following conditions: 160 kVp, tube current 20 mA, dose rate 110 R/minute at the surface of the animal's skin, fixed filtration 4 mm glass and 5 mm oil, 0 mm added filtration, source to skin distance 11 cm, HVL of 5 mm of Al.

Thermal neutron exposures were conducted in the bio-medical facility of the Neely Nuclear Research Center at Georgia Tech. This facility is a shielded room approximately 10 feet by 12 feet inside and is shielded with two feet of barytes concrete along the sides. It is located opposite

Table 10. Schedule of Exposures of C57BL Inbred Mice to Acute Doses of 160 kVp X-Rays, Thermal, and Fast Neutrons

Dose (rad in air)	Number Exposed		Age at Exposure (weeks)	Number of Lenses Analyzed		Type of Radiation
	Male	Female		Male	Female	
0	17	23	20-24	23	40	None
360	10	25	20	13	31	x-rays
740	none	6	8	none	11	x-rays
740	18	16	20-24	10	17	x-rays
960	none	9	4-8	none	5	x-rays
960	7	8	24	5	10	x-rays
1480	17	none	24	none	none	x-rays
1480 (740 + 740)	3	5	22	none	4	x-rays
40	7	12	20-24	10	16	thermal neutrons
80	8	11	21-23	9	18	thermal neutrons
300	3	12	4	8	15	fast neutrons
Total	90	127	70	78	167	

Grand Total of Lenses = 63 Control lenses + 182 Irradiated lenses = 245 lenses

the thermal column of the Georgia Tech Research Reactor (GTRR). The facility is fitted with a bismuth gamma shield, a collimator, and shutter. Access to the bio-medical room is through a vertically moving, hydraulically operated shielded door. The bio-medical shutter is operated by means of a hydraulic cylinder which is capable of opening or closing in 20 seconds or less. Under normal operating conditions, the bio-medical facility provides a mixed beam of thermal neutrons and gamma rays with a very small fast neutron component.

Fast neutrons (mean energy about 1 MeV) were obtained from the fast reactor (HPRR) of the Health Physics Division of Oak Ridge National Laboratory. The reactor core is basically a right annulus of 90% by weight uranium (93.14% U-235) and 10% by weight molybdenum alloy, eight inches in outside diameter, two inches in inside diameter, and nine inches long, surrounding a two inch in diameter stainless steel rod.

Dosimetry and Exposure Conditions

The x-ray exposures were measured in roentgens at the position of the eye using a 250 R Victoreen condenser chamber and R-meter, calibrated to within ± 3% using the Fricke chemical dosimeter (Dr. Robert Fetner, personal communication). The exposure in R was converted to absorbed dose of rads in air by multiplying the dose in roentgens by 0.869. Mice and gerbils were shielded except for the head with 3/4 inch of lead. The maximum whole-body exposure under these conditions was less than 5 R. The dose due to scattered radiation was determined also to be a negligible component of the total dose. The x-ray machine was operated remotely from an adjoining room during the exposures.

The mice which were irradiated in the bio-medical facility of the GTRR were shielded, except for the head, with 0.020 inch of cadmium and 3/4 inch of lead. They were irradiated in pairs with the body normal to the central axis of the beam port, at a distance of 15 inches from the port. The thermal neutron flux at the position of the eyes was measured with gold foils (both bare gold and gold covered with 0.019 inch of cadmium) and was found to be 4×10^8 n/cm^2-sec. The first collision dose rate for thermal neutrons at this position was determined to be 0.011 rad/sec, based on a conversion factor of 0.0272 ergs/gram-sec per 10^7 n/cm^2 incident on tissue-equivalent material (Bach and Caswell, 1968). The gamma ray absorbed dose rate was measured at the irradiation position using calibrated CaF TLD's and was 0.16 rad/sec. The gamma ray component accounted for about 94% of the total rad dose at the irradiation position of the head, indicating the high degree of gamma contamination of the thermal neutron beam in the bio-medical facility.

Also sulphur pellets were placed at the position of the eyes in the bio-medical facility in an attempt to evaluate the contribution from fast neutrons to the total lens dose during the GTRR irradiations. The $^{32}S(n,p)^{32}P$ reaction has a threshold for fast neutrons of about 3 MeV and an effective cross section of 310 millibarns. Twenty-four hours after irradiation of the pellets the induced ^{32}P beta ray activity was counted for 20 minutes on a Beckman low-beta proportional counter with an overall efficiency of 36% for the beta rays of ^{32}P. In this manner, the total fast neutron flux of neutrons of energy greater than 3 MeV was found to be 1.1×10^5 n/cm^2-sec, or about three orders of magnitude less

than the thermal flux. Thus, even allowing for a quality factor of ten for fast neutrons, it would seem that the fast neutron contribution was a very small fraction of the dose equivalent in the lens in this experiment.

Reactor power level during all exposures of mice in the biomedical room was 1,000 kW. The exposure conditions were identical for all animals.

In order to evaluate the effects of high doses of fast neutrons on the ionic composition of the mouse lens, a number of mice were exposed to the fast reactor (HPRR) of the Health Physics Division of Oak Ridge National Laboratory. The mice were not anesthetized, but were restrained in conical polypropylene tubes one millimeter in thickness. The reactor was operated at a steady state power level of 4 kW. The animals were given acute exposures (474 seconds in duration) of 512 rad in air, which after attenuation through the restraining tubes was equivalent to 300 rad in the mouse lens (L. W. Gilley). Exposures were conducted with the mice positioned in a radial array two meters from the reactor. The whole body was protected by a wedge-shaped shield consisting of borated paraffin, 60-70% boron by weight, and 1/2 inch of lead.

The fast neutron dose to the mouse lens was 300 ± 2 rad (M. C. Jernigan). The dose was calibrated by counting sulfur pellets which were irradiated along with each group of mice. At two meters from the HPRR reactor, gamma rays accounted for 14% of the dose in air, giving a neutron to gamma dose ratio of about six. The protective shield limited

the whole-body tissue dose as measured in air to be about 72 rad. The neutron energy spectrum of the HPRR has been calculated and measured by the Oak Ridge group and is shown in Figure 4 (L. W. Gilley).

Lens Examination

At three, six, and ten months after exposure, animals from each exposure group were examined by a trained ophthalmologist with an American Optical^{R} slit-lamp biomicroscope and then sacrificed in a chloroform box. The pupils were dilated prior to examination with 1% Isopto^{R} Atropine (atropine sulfate ophthalmic solution). The animals were fully awake during the examination. After sacrificing the animals, the lenses were removed immediately under a disecting microscope, rinsed with distilled water and frozen in polyethylene vials until neutron activation. All lenses were weighed prior to freezing and the wet weight recorded. The majority of adult mouse lenses in this project weighed about 7 mg. Young lenses were much more fragile than older lenses and weighed as little as 2 mg. The lenses were gently rolled on Kimwipes^{R} disposable wipers after enucleation from the eye to remove any adhering vitreous humor. Special care was taken to minimize lens contamination and to keep the lens capsule undamaged and intact. These lenses fractured quite easily and only whole lenses were used.

Activation Analysis of Lenses

Prior to activation, control and irradiated lenses were heat-sealed in polyethylene irradiation vials. The polyvials, together with 0.17% by weight cobalt-aluminum wire flux monitors, were sealed in

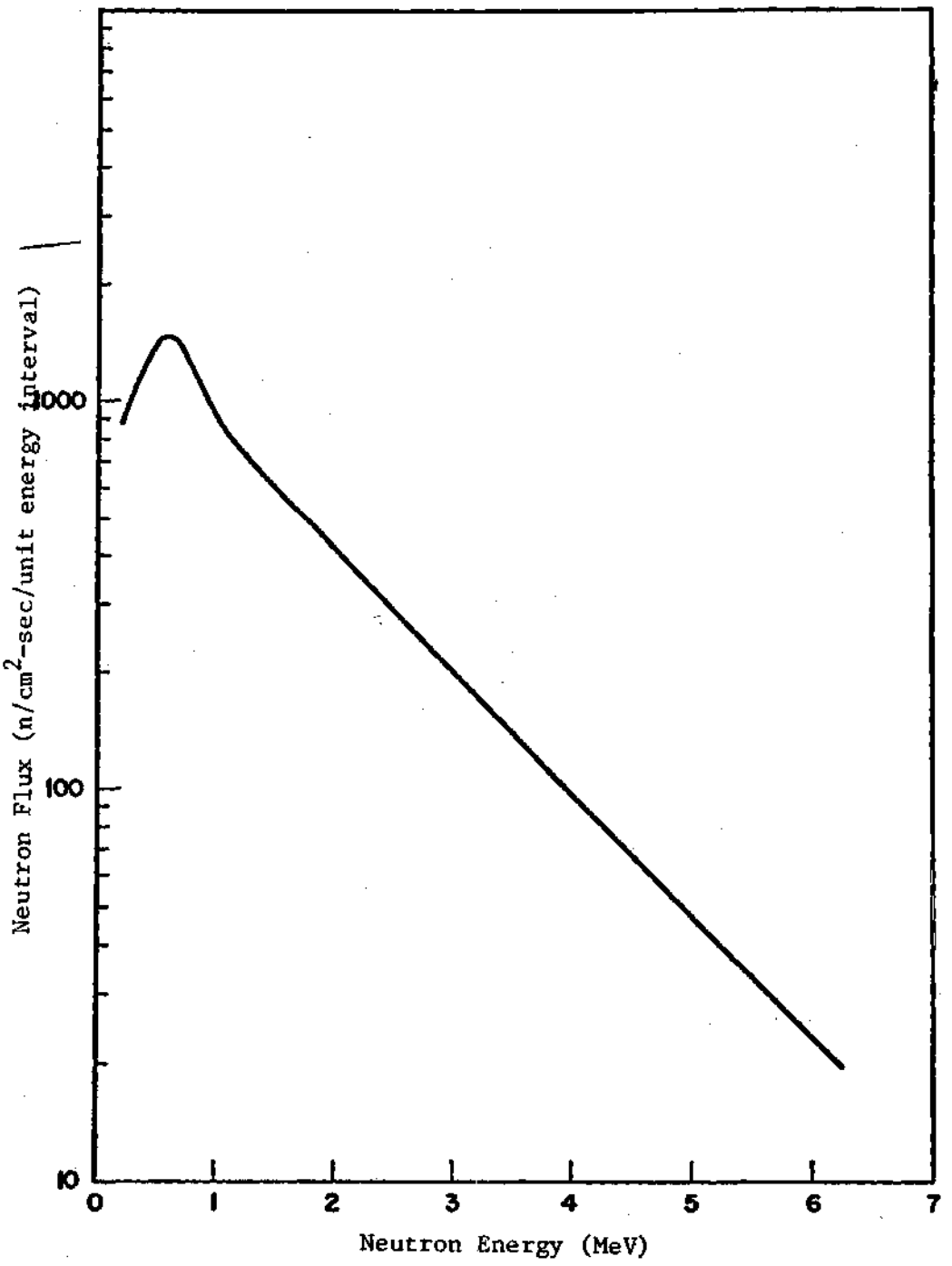


Figure 4. Neutron Energy Spectrum of HPRR (L. W. Gilley)

polybags which were then encapsulated in either polyethylene bottles or aluminum cans and activated in the GTRR, a fully-enriched, five megawatt, heavy-water moderated facility, for periods varying from two minutes to 55 hours. The wire flux monitors were wrapped around the polyvials to insure that both lenses and wires were exposed to as nearly the same thermal neutron flux as possible. The wire monitors did not appreciably affect the thermal neutron flux in the lenses. With the exception of one 55 hour run at 5,000 kW, reactor power level during activation was 1,000 kW. The thermal neutron flux at the activation positions (H-15, the pneumatic rabbit system; and V-21, a vertical sample tube) was about 10^{13} n/cm^2-sec.

After activation the lenses were transferred to new polyvials (to avoid counting induced activity in the vial itself) and counted for induced gamma ray activity on an Ortec lithium-drifted coaxial germanium detector with 6.8% relative efficiency and resolution 2.43 keV at the 1332 keV peak of Co-60. The detector had an active volume of 38.75 cm^3. The effective efficiency of this detector was determined using a National Bureau of Standards mixed gamma ray point source standard prepared at the NBS Center for Radiation Research in Washington, D. C. This standard consisted of cobalt-57, cobalt-60, strontium-85, yttrium-88, cadmium-109, tin-113-indium-113m, cesium-137-barium-137m, cerium-139, and mercury-203, deposited as the chlorides and sulfides on polyester tape. Gamma ray spectra were analyzed using a Nuclear Data 1024 multi-channel analyzer and displayed on a Tektronix Type R561B oscilloscope.

Efficiency data and curves for the Ge-Li detector obtained by

using the NBS standard are presented in Table 11 and Figure 5, respectively. A representative background for this detector is given also in Table 12. The K-40 (1461.2 keV) was the predominant peak in the gamma ray background spectrum and probably originated from walls of the counting room and inside the detector crystal.

The net counts under each gamma ray peak were calculated by a Hewlett-Packard 9100B calculator interfaced with the multi-channel analyzer. The energy calibration program of the HP-9100B computer provided a method of measuring the energy calibration of the multi-channel analyzer in terms of energy per channel and zero offset energy. The centroids of the peaks for two separate gamma ray peaks of known energy (the 661.635 keV peak of Cs-137 and the 1332.49 keV peak of Co-60) were used in the energy calibration. A separate program identified as Peak Picker located the peaks in the gamma ray spectrum and provided energy and net count information for each peak. The program identified a peak about channel "i" by determining that the number of counts N_i in channel "i" was greater than the number of counts in channels (i-2) and (i+2) by an adjustable parameter P times the standard deviation in the number of counts in channels (i-2) and (i+2), respectively. In other words, two tests had to be satisfied:

$$(N_i - N_{i-2}) > P\sqrt{N_{i-2}} \quad \text{and} \quad (N_i - N_{i+2}) > P\sqrt{N_{i+2}}$$

Values of P from 5 to 10 were used. The adjustable parameter P simply allowed the investigator to evaluate the reliability of the counting data. The greater the value of P that was used the more reliable was the peak information calculated by the program. When both of the

Table 11. Effective Efficiency, ε, of Nuclear Research Center Ge-Li Detector at Various Locations as a Function of Gamma Ray Energy†

Gamma Energy (keV)	Source Strength* (photons/sec)	Effective Efficiency, ε		
		Detector Face	Shelf #1	Shelf #2
88	850.7	0.1632	0.0761	0.0250
122	720.6	0.1782	0.0724	0.0237
165	254.9	0.1248	0.0220	0.0169
392	313.8	0.0481	0.0166	0.0061
662	6,496.8	0.0235	0.0088	0.0032
898	1,252.2	0.0161	0.0060	0.0022
1173	11,678.0	0.0106	0.0044	0.0016
1333	11,686.0	0.0095	0.0038	0.0014
1836	1,332.1	0.0064	0.0027	0.0010

†Samples and standards were counted under identical conditions of geometry on various levels of a Lucite® stand, 0.060 inch in thickness, along the central axis of the detector. Shelf #1 and #2 were 1/2 inch and 1 1/2 inches from the detector face, respectively.

*Using NBS Mixed Gamma-Ray Point-Source Standard # SRM 4215-B, consisting of the nuclides cobalt-57, cobalt-60, strontium-85, yttrium-88, cadmium-109, tin-113-indium-113m, cesium-137-barium-137m, cerium-139, and mercury-203, deposited as the chlorides and sulfides on polyester tape.

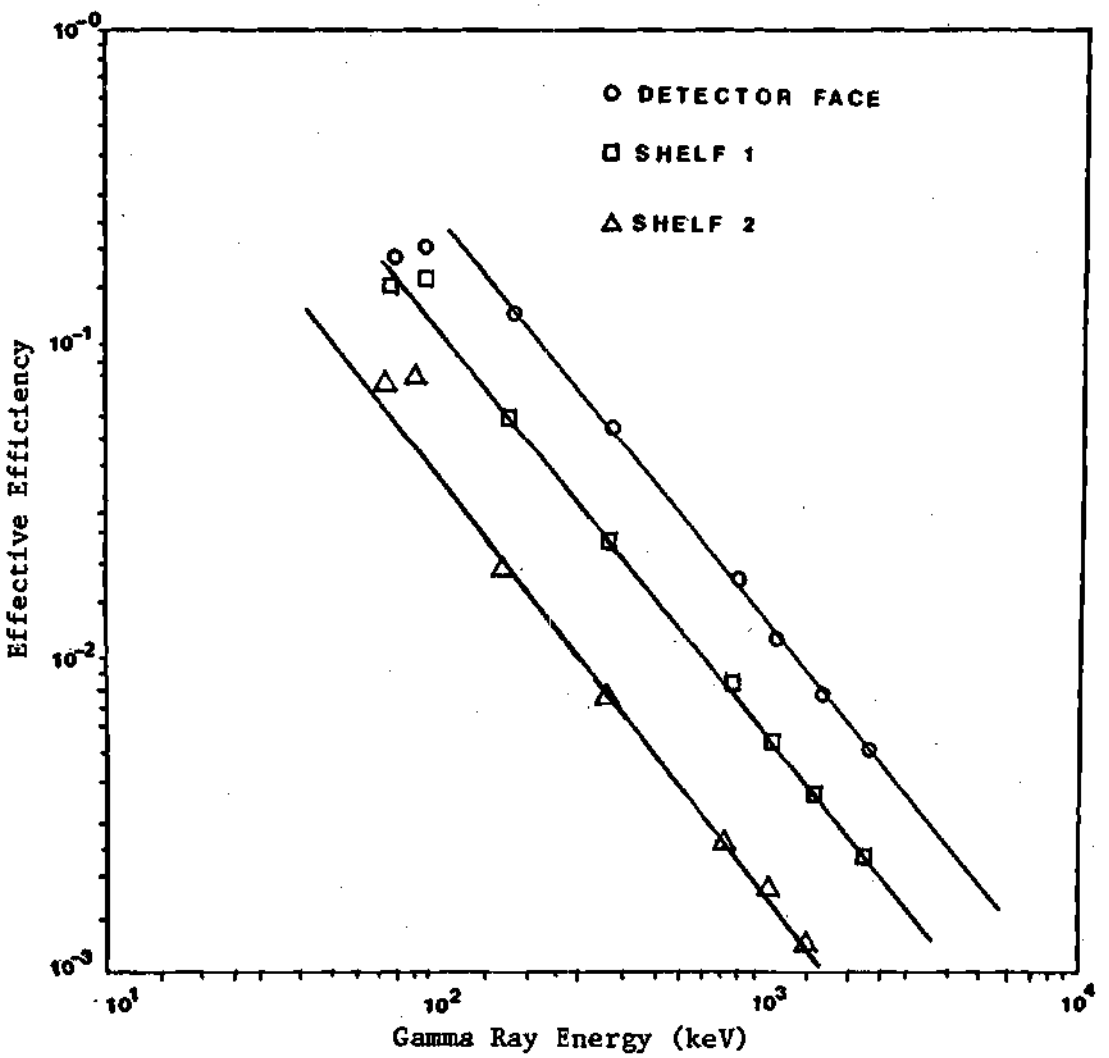


Figure 5. Effective Efficiency vs Gamma Ray Energy for Ge(Li) Detector

Table 12. Ge(Li) Detector Background

Energy (keV)	Nuclide	Count Rate* (cps)
239.9	Pb-212	0.104 ± 0.001
295.6	Pb-214	0.014 ± 0.0004
338.9	Ac-228	0.016 ± 0.0004
352.8	Pb-214	0.044 ± 0.0007
511.1	Tl-208 + Rn-222	0.043 ± 0.0007
582.6	Tl-208	0.035 ± 0.0007
609.8	Bi-214	0.041 ± 0.0007
728.1	Bi-212	0.009 ± 0.0004
769.6	Bi-214	0.002 ± 0.0002
861.2	Tl-208	0.005 ± 0.0003
910.8	Ac-228	0.028 ± 0.0006
969.5	Ac-228	0.008 ± 0.0003
1120.4	Bi-214	0.011 ± 0.0004
1238.9	Bi-214	0.006 ± 0.0003
1379.5	Bi-214	0.002 ± 0.0002
1461.2	K-40	0.114 ± 0.001
1592.8	Ac-228	0.007 ± 0.0003
1765.1	Bi-214	0.009 ± 0.0004
1848.3	Bi-214	0.001 ± 0.0001

*An 80,000 second live time count inside the lead brick shield. Count rates are given as counts per second ± the square root of the total number of counts divided by the counting time in seconds.

above tests were satisfied, the centroid of the peak channel was calculated and converted to gamma ray energy using previously stored energy calibration data. The net count under each peak was calculated also using channels (i-2) and (+2) for baseline end points, and the standard deviation N_{i-2} was calculated and displayed to allow evaluation of the validity of the net count data. This test was repeated until the entire gamma ray spectrum was scanned. The count rates in this thesis were high enough that the value of P entered into the program had no effect on the ultimate results. The counting error in this investigation was adjusted to be less than ± 5% by accumulating counts until the net counts under each unknown gamma ray peak exceeded 1,000.

Concentrations of sodium, potassium, chlorine, and manganese in control and experimental lenses were calculated using the single-comparator method (Simonits, De Corte, and Hoste, 1975). The term single-comparator refers to a technique where multi-element analysis can be performed by irradiating and measuring a single element (comparator) instead of using standards prepared from known weights of the elements to be determined. The specific activities of the standards (A_{sp}) can be calculated by multiplying the measured specific activity of the comparator (A^*_{sp}) by the experimentally-determined k-factors, which can be written as:

$$k = \frac{A_{sp}}{A^*_{sp}} = \frac{M^* \cdot \gamma \cdot \epsilon_p \cdot \theta \cdot \hat{\phi} \cdot \hat{\sigma}}{M \cdot \gamma^* \cdot \epsilon^*_p \cdot \theta^* \cdot \hat{\phi}^* \cdot \hat{\sigma}^*} \qquad (1)$$

with

$$A_{sp} = \frac{A_p}{S\ D\ C\ w} \qquad (2)$$

where M = atomic weight of the irradiated element

θ = isotopic abundance of the target nuclide

γ = absolute abundance of the measured gamma-ray

ϵ_p = full-energy peak efficiency of the detector for the measured gamma-ray energy

$\hat{\phi}$ = conventional reactor neutron flux in n/cm^2-sec

$\hat{\sigma}$ = effective reactor neutron cross-section in barns

$S = [1 - \exp(-\lambda t_{irr})]$, a factor dependent on decay constants and irradiation time, t_{irr}

$D = \exp(-\lambda t_d)$, the decay factor where t_d is the decay period

$C = (1 - \exp - \lambda t_m)/\lambda t_m$, the measurement factor correcting for decay during the counting period t_m

A_p = measured average intensity of full-energy peak in counts/sec

w = weight of the element in grams.

The weight of the unknown element in the lens was then given by the following formula:

$$W_u = \frac{A_u}{kA_{comp}} \tag{3}$$

where W_u = weight of the unknown element in grams

A_u = saturation activity of the unknown element in counts/sec

k = k-factor for the particular element (no units)

A_{comp} = saturation activity of the Co-Al comparator wire in counts/sec per gram of cobalt.

The comparator used in this study was high-purity flux wire,

0.020 inch in diameter, composed of 0.17% by weight cobalt in aluminum. Cobalt has nuclear parameters which make it ideal for the measurement of the high neutron fluxes which occur in reactors. It is a 1/v-detector and has a g-factor of unity. The dilution of the cobalt monitor in aluminum minimizes errors in flux measurement due to self-shielding by the cobalt comparator, provided enough time is allowed for full decay of the Al-28 activity.

Once the k-factors are determined, the preparation of standards can be eliminated in any further analysis. It was assumed that the k-factors are constant in time; the accuracy of the method, therefore, depends mainly on the constancy of the k-factors. The set of k values is valid only for one fixed sample-detector distance, so that ϵ_p/ϵ_p^* in Equation (1) remains constant. Consequently, the standards, samples, and comparator must have constant geometric form during the entire set of experiments. Also, because the $\hat{\sigma}/\hat{\sigma}^*$ effective cross-section ratio depends on the reactor spectrum (i.e., mainly on $\hat{\phi}_{th}/\hat{\phi}_e$, the thermal to epithermal flux ratio), the set of k-values is valid only for one selected irradiation position. Moreover, if any change occurs in the ratio $\hat{\phi}_{th}/\hat{\phi}_e$, due to reactor core modifications, the whole set of k-factors must be remeasured. That for a fixed reactor core design the ratio of $\hat{\phi}_{th}$ to $\hat{\phi}_e$ remains constant we cite the example of the "Thetis" reactor of Ghent University, where the flux-ratios (ranging from 20 to 200) in a number of irradiation channels remained constant within ± 2% during the period 1971-1973 (Simonits, De Corte, and Hoste, 1975).

De Corte et al. (1969) showed that the k-factors determined in

one reference channel (where the ratio $\hat{\phi}_{th}/\hat{\phi}_e$ is well known) can be re-evaluated for any other channel by the following equation:

$$k_{anal} = \frac{k_{ref} \cdot \left[\left(\frac{\hat{\phi}_{th}}{\hat{\phi}_e}\right)_{anal} + \left(\frac{I_o}{\hat{\sigma}_{th}}\right)_{st}\right]\left[\left(\frac{\hat{\phi}_{th}}{\hat{\phi}_e}\right)_{ref} + \left(\frac{I_o}{\hat{\sigma}_{th}}\right)_{comp}\right]}{\left[\left(\frac{\hat{\phi}_{th}}{\hat{\phi}_e}\right)_{anal} + \left(\frac{I_o}{\hat{\sigma}_{th}}\right)_{comp}\right]\left[\left(\frac{\hat{\phi}_{th}}{\hat{\phi}_e}\right)_{ref} + \left(\frac{I_o}{\hat{\sigma}_{th}}\right)_{st}\right]} \qquad (4)$$

where $\hat{\phi}_{th}$ = conventional thermal (subcadmium) neutron flux

$\hat{\sigma}_{th}$ = thermal neutron cross section

$\hat{\phi}_e$ = epithermal neutron flux, assumed to follow the 1/E shape, so $\phi_e(E) = \phi_e/E$

$I_o = \int_{E_{Cd}}^{\infty} (E)dE/E$, the infinitely dilute resonance integral

and anal., st., and comp. represent analysis channel, standard, and comparator, respectively. The effective Cd cutoff energy for small 1/v detectors was assumed to be 0.55 eV.

To determine the single comparator k-factors for the elements Na, K, Cl, and Mn in various irradiation channels in the GTRR, standards were prepared from certified reagents: sodium chloride, anhydrous potassium nitrate, and hydrated manganous chloride. These reagents had less than 0.003% insoluble matter and less than 0.0005% sulfate (SO_4) and heavy metals (Pb and Fe) as impurities. Polyvials were leached for one hour in 1 NaOH, rinsed in water and alcohol, and then dried. Leaching did not introduce appreciable amounts of contaminants (M. Bruce). Small amounts of each reagent were carefully weighed and then diluted in distilled, de-ionized water so that the concentrations of the standards

were of the same order of magnitude as that of the respective unknowns in the lens of the mouse and gerbil eye. A blank consisting of distilled, de-ionized water in a polyvial was activated also along with the standards. Thus, three standards and one blank were activated, along with two Co-Al flux wire comparators (total weight: 95.0 and 93.8 mg), in a polyethylene bottle for ten minutes in the pneumatic tube (H-15) of the GTRR.

The k-factors for each of the four standards were then determined for various counting geometries on the Nuclear Research Center Ge(Li) semiconductor detector inside a lead brick shield. The standards were counted on various shelves of a rectangular Lucite^{R} stand which fitted over the detector and insured reproducible counting geometry. This stand was 0.060 inch thick at the base and did not significantly attenuate the gamma radiation from the standards and samples.

The single comparator k-factors for sample irradiation in the pneumatic tube (H-15) of the Georgia Tech Research Reactor are shown in Table 13. The corresponding k-factors for sample irradiation in V-22 are presented in Table 14. These k-factors were calculated from those measured in H-15 using Equation (4) and the thermal to epithermal flux ratios in the two channels. The $\hat{\phi}_{th}/\hat{\phi}_{e}$ was about 26 in H-15 and three in V-22.

Error Analysis

As mentioned earlier, counting errors were adjusted to be less than ± 5% by accumulating counts until the net counts under each unknown

Table 13. Single-Comparator* k-Factors for Sample Irradiation in H-15, the Pneumatic Tube, of Georgia Tech Research Reactor

	Weight (g)	Specific Activity (cps/g element or solution) Det. Face**	Shelf #1	Shelf #2	k-Factor Det. Face	Shelf #1	Shelf #2
Standard							
Na	$2.04 \cdot 10^{-4}$	$5.05 \cdot 10^{8}$	$2.13 \cdot 10^{8}$	$7.84 \cdot 10^{7}$	$4.65 \cdot 10^{-2}$	$3.39 \cdot 10^{-2}$	$2.79 \cdot 10^{-2}$
K	$1.89 \cdot 10^{-3}$	$1.01 \cdot 10^{7}$	$2.90 \cdot 10^{6}$	$1.61 \cdot 10^{6}$	$9.26 \cdot 10^{-4}$	$4.63 \cdot 10^{-4}$	$5.72 \cdot 10^{-4}$
Mn	$2.43 \cdot 10^{-7}$	$4.29 \cdot 10^{9}$	$2.57 \cdot 10^{9}$	$1.18 \cdot 10^{9}$	$3.95 \cdot 10^{-1}$	$4.09 \cdot 10^{-1}$	$4.20 \cdot 10^{-1}$
Cl	$3.14 \cdot 10^{-4}$	---	---	$3.27 \cdot 10^{6}$	---	---	$1.16 \cdot 10^{-3}$
Co-Al Wire #1	$1.62 \cdot 10^{-4}$	$1.13 \cdot 10^{10}$	$6.30 \cdot 10^{9}$	$2.75 \cdot 10^{9}$	---	---	---
Co-Al Wire #2	$1.59 \cdot 10^{-4}$	$1.04 \cdot 10^{10}$	$6.25 \cdot 10^{9}$	$2.87 \cdot 10^{9}$	---	---	---
Blank	0.892						
Na	---	$9.79 \cdot 10^{1}$	$5.79 \cdot 10^{1}$	$2.85 \cdot 10^{1}$	---	---	---
K	---	---	---	---	---	---	---
Mn	---	$2.92 \cdot 10^{1}$	$1.55 \cdot 10^{1}$	$6.97 \cdot 10^{0}$	---	---	---
Cl	---	$3.48 \cdot 10^{1}$	$2.30 \cdot 10^{1}$	$8.34 \cdot 10^{0}$	---	---	---

*Using 0.17% by weight Co-Al flux wire as a single-comparator.

**Along central axis of Neely Nuclear Research Center Ge-Li semiconductor detector (6.8% relative efficiency and resolution 2.43 keV at the 1332 keV peak of Co-60; active volume = 38.75 cm^3).

Table 14. Single Comparator k-Factors for Sample Irradiation in V-22 of Georgia Tech Research Reactor*

	Detector Face	Shelf #1	Shelf #2
Na	$3.36 \cdot 10^{-2}$	$2.45 \cdot 10^{-2}$	$2.01 \cdot 10^{-2}$
K	$7.59 \cdot 10^{-4}$	$3.79 \cdot 10^{-4}$	$4.68 \cdot 10^{-4}$
Mn	$3.17 \cdot 10^{-1}$	$3.28 \cdot 10^{-1}$	$3.37 \cdot 10^{-1}$
Cl	--	--	$8.99 \cdot 10^{-4}$

*Based on an eight hour irradiation at 1,000 kW. The k-factors were measured along the central axis of the Ge(Li) detector (6.8% relative efficiency and resolution 2.43 keV at the 1332 keV peak of Co-60). The detector had an active volume of 38.75 cm^3. Shelf #1 and #2 were 1/2 inch and 1 1/2 inches from the detector face, respectively.

gamma ray peak had exceeded 1,000. Actually in most cases the error due to uncertainty in net counts under each peak was less than ± 1%.

One source of error was the positioning of lenses in new polyvials for counting after activation. It was necessary to transfer the lenses since the polyvials themselves had appreciable induced activities of sodium and chlorine. Every effort was made to place each group of lenses in the same location in the counting vials.

Lenses were weighed to ± 0.05 mg using a precision Metler balance. Since the average weight of the lenses used in this experiment was about 7 mg, this error amounted to about 1%. Small fluctuations in true lens weight may have resulted due to variation in lens moisture content while exposed to the air.

Any errors in the timing of activation in the reactor are considered quite small. The pneumatic tube is capable of inserting samples into the reactor core in less than one second and all activation experiments were at least 120 seconds in duration.

Another source of error in the activation experiments is variation in the thermal neutron flux at the activation positions within the reactor. Comparison of the specific activities of the flux wires from each separate experiment indicated that the thermal neutron flux levels in the pneumatic tube vary no more than ± 15%, at a steady power level of 1,000 kW.

CHAPTER VII

RESULTS AND DISCUSSION

The Mongolian Gerbil

Table 15 lists the nuclides which were detected in the gerbil lens following activation by thermal neutrons. The predominant elements observed in the lens following activation by thermal neutrons were sodium, potassium, chlorine and manganese. In addition the following trace elements were detected after long (eight hours or greater) reactor irradiation of lenses: selenium, gold, bromine, rubidium, zinc, cobalt, antimony, chromium and scandium. The principal peaks in the gamma ray spectrum of normal adult whole eyes of the Mongolian gerbil are presented by increasing gamma ray energy in Table 16.

No changes in lens appearance as observed by slit-lamp were found in gerbils as late as nine months after an acute exposure to 1,000 R of 160 kVp x-rays. However, nine months after x-ray exposure slight but measurable changes in sodium and chlorine concentrations were detected in lenses of these exposed animals. Table 17 summarizes the data for gerbil lenses and presents normal ion concentrations in lenses of selected mammals for comparison with gerbil lenses, both exposed and unexposed to x-rays. Sodium levels rose from 17.5 ± 1.1 mg Na per 100 grams lens wet weight in controls to 22.0 ± 1.2 mg per 100 grams lens wet weight in irradiated lenses. Chlorine levels rose from 19.9 ± 2.1 mg Cl per 100 grams lens wet weight in controls to 31.3 ± 1.2 mg per 1000 grams lens wet weight

Table 15. Parameters of Nuclides Observed in Lenses of Mice and Mongolian Gerbils Following Thermal Neutron Activation in Order of Ascending Gamma Ray Energy

Nuclide	Principal Gamma Ray Energy (keV)	Cross Section (barns)	Half-Life
Se-75	265	30	120 d
Cr-51	320	17	27.8 d
Au-198	412	98.9	2.7 d
Sb-122	564	6.1	2.8 d
Br-80	666	8.5	18 min
Br-82	776	3	35 hr
Mn-56	848	13.3	2.6 hr
Sc-46	889	23	83.9 d
Rb-86	1077	1.0	18.7 d
Zn-65	1116	0.46	245 d
Co-60	1173	35	5.2 yr
Na-24	1369	0.53	15.0 hr
K-42	1525	1.2	12.4 hr
Cl-38	1642	0.40	37 min

Table 16. Observed Peaks in the Gamma Ray Spectrum of Normal Adult Eyes of Mongolian Gerbils Following Activation with Thermal Neutrons (in order of ascending energy)

Energy (keV)	Nuclide	Energy (keV)	Nuclide
123.3	Se-75	1,116.6	Zn-65
137.3	Se-75	1,146.0	Cl-38 DEP†
221.5	Br-82	1,173.8	Br-82 SP‡
265.8	Se-75	1,253.6	Br-82 SP
273.4	Br-82	1,318.5	Br-82
281.5	Se-75	1,331.0	Br-82 SP
312.8	K-42	1,369.5	Na-24
511.0	Cu-64	1,395.9	Br-82 SP
555.0	B4-82	1,476.9	Br-82
616.1	Br-80	1,524.4	K-42
619.9	Br-82	1,605.4	Br-82 SP
665.5	Br-80	1,642.0	Cl-38
699.7	Br-82	1,657.7	Cl-38 SEP
777.1	Br-82	1,733.2	Na-24 DEP
828.2	Br-82	1,810.4	Mn-56
846.0	Mn-56	1,872.4	Br-82 SP
857.0	Na-24 SEP*	2,032.1	Br-82 SP
1,009.0	Br-82	2,096.9	Br-82 SP
1,044.1	Br-82	2,168.3	Cl-38
1,077.2	Rb-86	2,244.2	Na-24 SEP

*SEP indicates a single escape peak. †DEP is a double escape peak. ‡SP indicates a sum peak. The underlined energies indicate the principal peaks in the spectrum.

Table 17. Ion Concentrations[†] in Lenses of Mongolian Gerbils and Selected Mammals

Lens Type	Sodium	Chlorine	Potassium	Manganese	K/Na	Selenium	Zinc	Rubidium
Gerbil Control (30)[‡]	17.5 ± 1.13	19.9 ± 2.08	212.1 ± 15.1	0.0183 ± 0.0067	12.1	0.962 ± 0.361	4.00 ± 0.97	1.86 ± 1.03
Gerbil Exposed (31)[‡]	22.0 ± 1.23	31.3 ± 1.21	238.5 ± 9.19	0.00975 ± 0.00189	10.8	---	Not Measured	---
Human[*]	91.0	35.3	170.0	---	1.9	---	---	---
Rat[*]	78.0	29.0	222.0	---	2.8	---	---	---
Rabbit[*]	59.0	35.0	472.0	---	8	---	---	---
Cattle[*]	46.0	69.0	404.0	0.012	8.8	---	1.5	---

[†]Mean values and their standard errors of ion concentrations are given as milligrams of ion per 100 grams of lens wet weight. [*]Taken from Kuck (1970). [‡]Numbers in parentheses indicate total number of lenses analyzed in this study. Mongolian gerbils 12 months of age were given single acute doses of 1,000 R of 160 kVp x-rays and the lenses enucleated nine months after exposure. No cataracts were observed as late as nine months after exposure.

in irradiated lenses (1,000 R of 160 kVp x-rays in a single acute exposure). The potassium to sodium ratio in gerbils exposed to 1,000 R decreased to 89% of normal, that is, from 12.1 to 10.8.

It is apparent that the normal potassium to sodium ratio in the lens of the Mongolian gerbil is higher than that of other mammals and is about six times that in man. This may reflect their desert habitat and low water uptake. The potassium concentration found in the gerbil lens is about the same as that reported for man and the rat (Kuck, 1970). The sodium and chlorine concentrations, however, appear to be somewhat lower than those in the mammals used for comparison. The relatively high K/Na ratio in the gerbil lens could be a factor in their radiation resistance and it would be interesting to measure this ratio in other tissues of the gerbil such as skin and brain. Marked changes in whole-body exchangeable sodium and potassium levels have been observed in patients treated with ionizing radiation (Olson et al., 1970). A decrease in the K/Na ratio was observed during radiation therapy which was correlated with field size and integral dose.

Results from Experiments with Mice

Clinical Observations

The observed radiation effects on C57BL inbred black mice and Mongolian gerbils are presented in Table 18. The 17 mice which were exposed to an acute dose of 1,480 rad of 160 kVp x-rays developed a white mucus in their eyes about eight to nine days after exposure. Two weeks after irradiation the surviving animals had complete epilation of the face with swelling of the eyes. Many animals had developed either dry or wet desqua-

Table 18. General Radiation Effects on C57BL Inbred Black Mice and Mongolian Gerbils

Dose (rad)	Age Group (weeks)	Type of Radiation	Time to Cataract	Optical Effects	K/Na
Exposed Mice					
0	4-8, 20-24	None	Not observed	None	3.6
40	20-24	Thermal neutrons	Not observed	Corneal scarring	3.6
80	20-24	Thermal neutrons	Not observed	Corneal scarring	3.6
300	4	Fast neutrons	2 1/2 months	Cataracts	2.6
360	20-24	160 kVp x-rays	Not observed	Lenses clear	2.1
740	4-8	160 kVp x-rays	3 months	cataracts; corneal keratitis	0.22
740	20-24	160 kVp x-rays	10 months	cataracts; corneal keratitis	0.60
960	4-8	160 kVp x-rays	3 months	cataracts; corneal keratitis; microphthalmia	0.11
960	20-24	160 kVp x-rays	10 months	cataracts; corneal keratitis; microphthalmia; anophthalmia	0.43
1480 (740 + 740)	20-24	160 kVp x-rays	Not observed	corneal vascularization; microphthalmia; anophthalmia	0.19
1480	20-24	160 kVp x-rays	Not observed	anophthalmia; microphthalmia	----

Table 18. Continued

Dose (rad)	Age Group (weeks)	Type of Radiation	Time to Cataract	Optical Effects	K/Na
Exposed Gerbils					
0	50	None	Not observed	None	12.1
1,000 (gerbils)	50	160 kVp x-rays	Not observed	No effects	10.8

NOTE: Mice and Mongolian gerbils were given single acute exposures of 160 kVp x-rays, thermal or fast neutrons and the lenses enucleated at various times up to 10 months post-exposure. The lenses were examined by slit-lamp biomicroscope at 3, 6 and 10 months after irradiation. One group of eight mice was given two acute exposures of 740 rad each of 160 kVp x-rays for a total of 1,480 rad. Gerbils were exposed to an acute dose of 1,000 R of 160 kVp x-rays in a single brief exposure. The K/Na ratio refers to that in the whole lens.

mation of the face and epilation on the underneath portion of the body extending to the armpits. The skin under the neck was observed to be very pink and tender to the touch. The eyes were also highly sensitive to light. By one month after exposure 41% of the animals in this group had died. Most of the animals had total eye degeneration with rupture of the eyeball. None of these mice survived more than two months after irradiation.

In contrast, mice which received 1,480 rad of x-rays in two separate fractions of 740 rad spaced three days apart survived until the time of sacrifice (five months). Epilation did occur but was less severe in this group than in animals receiving a single acute dose of 1,480 rad. Some bleeding and necrosis of the ears was noted in this group. Almost no epilation was observed on the underneath portions of the body. Three of the eight animals in this group exhibited slight exophthalmia.

Animals which received 740 rad in a single acute exposure did not epilate and exhibited excellent survival rates. The hair of the head turned snow white in appearance. Animals which received 360 rad had excellent survival rates and retained more of their black hair pigment than did animals exposed to 740 rad.

Mice which were exposed to 40 and 80 rad of thermal neutrons exhibited no visible systemic changes nor did they develop lens opacities in the period of study post-exposure. Mice exposed to 300 rad of fast neutrons to the lens exhibited mild depigmentation of body hair at two months post-exposure.

Cataract Formation

As shown in Table 18, cataracts in mice were observed at only two x-ray dose levels and in two different age groups. Radiation opacities detectable by skilled slit-lamp examination were observed first in the four to eight week old age group of mice exposed to 740 and 960 rad of x-rays and appeared about three and one-half months after exposure. The mice in the 740 rad group exhibited corneal opacity and keratitis in the nasal quadrant. One animal showed a posterior peripheral opacity three months post-irradiation. Mice in the 960 rad exposure group showed posterior cortical opacities with swollen lens nuclei. One mouse exhibited microphthalmia of the left eye and another microphthalmia in both eyes. These individuals also had corneal vascularization.

In contract to the four to eight week old mice which developed typical radiation opacities within three months after exposure, mice in the 20-24 week old group did not develop opacities detectable by slit-lamp up to 10 months after exposure to 740 and 960 rad.

Mice exposed to 360 rad of 160 kVp x-rays did not develop cataracts within the period of observation.

Mice exposed to 40 and 80 rad of thermal neutrons had not developed lens opacities as late as 10 months after exposure.

In the last column of Table 18 the K/Na ratios measured in the whole lens are presented for comparison with the incidence of cataract formation in mice. The first precipitous drop in the K/Na ratio occurred after an acute exposure of 360 rad of 160 kVp x-rays. Because cataracts were not observed at this dose level, it was not possible to establish a direct

relationship between significant changes in this ratio and probable cataract formation. In their studies of survival and cataract formation in Swiss mice, Riley and co-workers (1956) summarized opacification of the mouse lens one year after a single, acute exposure (dose rate 33 R per min) to 200 kVp x-rays. Lenses which had received 400 R of x-rays showed vacuoles in the subcapsular cortex of the posterior polar region one year after exposure. They observed that in the dose range from 100-500 R, the amount of damage to the lens increased slightly with increased exposure, and in none of the lenses in this dose range did cataracts develop which progressed to the more advanced stages.

K/Na Ratios

A comparison of the activation spectra of control and irradiated lenses is given in Figure 6. It should be noted that in the control spectrum the K-42 peak (which is directly proportional to the amount of potassium in the lenses analyzed) is above the K-40 background peak, whereas the K-42 peak in the irradiated lens spectrum has fallen below the background peak due to loss of potassium in lenses exposed to x-rays. Figure 7 is a difference spectrum obtained between control and x-irradiated mouse lenses.

Table 19 is a comparison of the activation spectra as measured by specific activities of control and x-irradiated mouse lenses following a 20 minute activation in the reactor in a thermal neutron flux of about 10^{13} n/cm^2-sec. Irradiated lenses were given two acute doses of 740 rad each of 160 kVp x-rays, spaced three days apart. The delay time between x-ray exposure and lens enucleation, after which neutron activation was

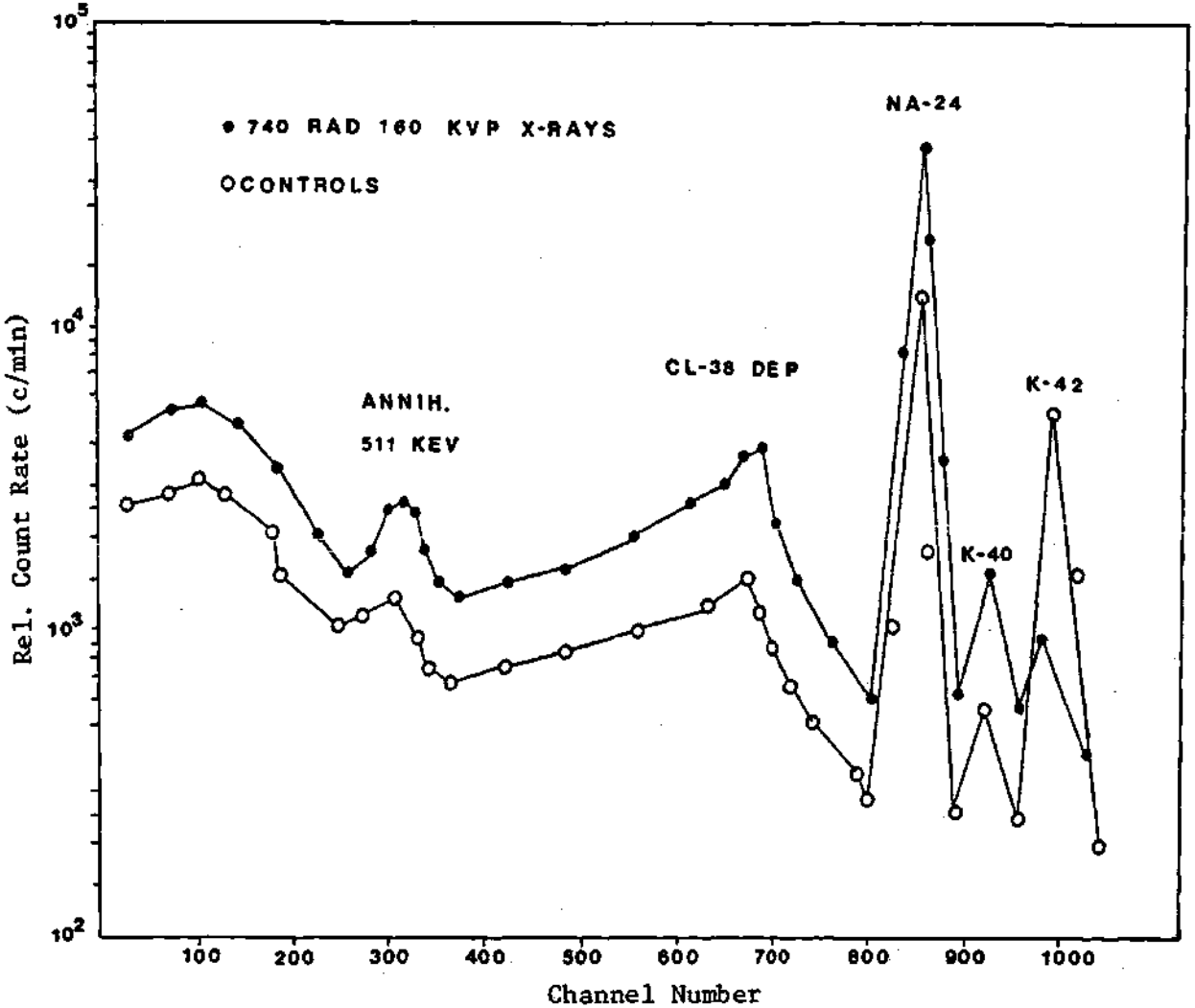


Figure 6. Comparison of Neutron Activation Spectra of Control and X-irradiated Adult Mouse Lenses

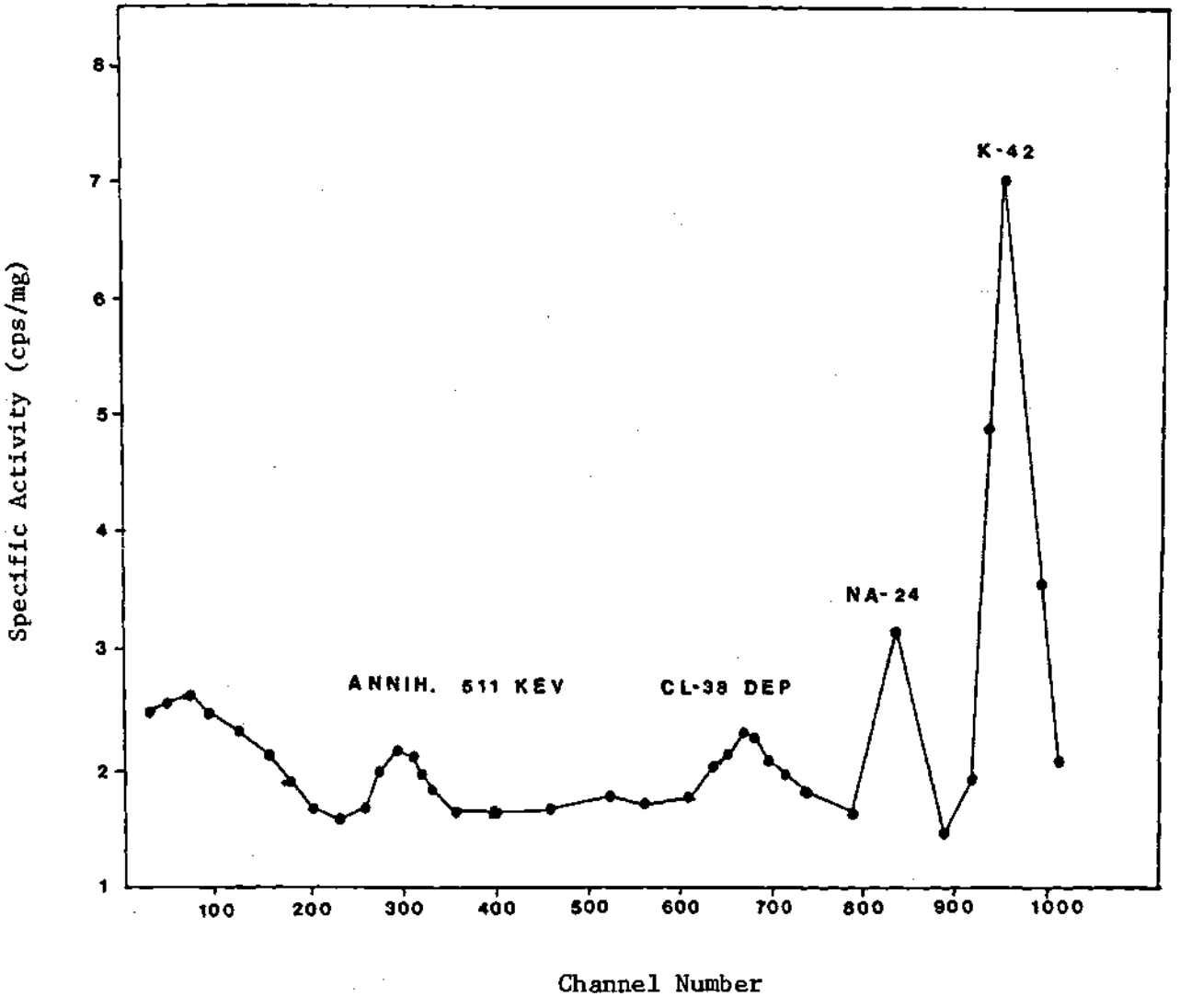


Figure 7. Difference Spectrum Obtained for Control and X-irradiated Adult Mouse Lenses

Table 19. Comparison of Activation Spectra of Control and X-irradiated Adult Mouse Lenses

Gamma Ray Energy (keV)	Nuclide	Specific Activity (cps/mg)	
		Controls	1,480 rad X-rays
347.4	Na-24 DEP†	$3.98 \cdot 10^{-2}$	$6.46 \cdot 10^{-2}$
510.9	Annih.	$1.60 \cdot 10^{-1}$	$3.27 \cdot 10^{-1}$
1,145.4	Cl-38 DEP	$7.23 \cdot 10^{-2}$	$15.4 \cdot 10^{-2}$
1,368.2	Na-24	$3.09 \cdot 10^{0}$	$6.46 \cdot 10^{0}$
1,524.4	K-42	$2.03 \cdot 10^{-1}$	$0.29 \cdot 10^{-1}$
1,642.3	Cl-38	$3.17 \cdot 10^{-1}$	$7.63 \cdot 10^{-1}$
1,656.9	Cl-38 SEP‡	$1.89 \cdot 10^{-2}$	---
1,731.9	Na-24 DEP	$6.27 \cdot 10^{-1}$	$12.9 \cdot 10^{-1}$

†Indicates a double escape peak.

‡Indicates a single escape peak.

Mouse lenses were given two acute doses of 740 rad each of 160 kVp x-rays in single brief exposures spaced three days apart. Six months after irradiation these lenses and control lenses of the same age were enucleated and then activated under identical conditions for 20 minutes in the pneumatic tube of the GTRR in a thermal neutron flux of about 10^{13} n/cm^2-sec. The activated lenses were then analyzed by gamma-ray spectroscopy using a Ge-Li detector.

carried out, was six months. No cataracts were observed at this time yet the specific activities of the peaks in the irradiated spectrum reflect higher concentrations of sodium and chlorine and much reduced (by a factor of seven) potassium levels as compared to controls.

The measurements show that as the normal unirradiated mouse lens ages it increases in wet weight and gradually suffers a net decrease in the potassium to sodium ratio in the whole lens (see Figure 8). The lenses in this experiment increased linearly in wet weight from about 1.5 mg at age three weeks to a maximum weight of about seven mg at age 28 weeks. On the other hand the K/Na ratio in the whole mouse lens decreased from 4.2 at two weeks of age to 3.5 at 12 months of age, or a reduction in the K/Na ratio of 17%. A similar decrease of 18% in the K/Na ratio in the whole ox lens was reported by Amoore et al. (Kuck, 1970). This trend is due mainly to a slight rise in lens sodium concentration rather than a loss of lens potassium.

Concentrations of sodium, potassium and chlorine in control and irradiated lenses six to nine months after acute exposure to 160 kVp x-rays, thermal or fast neutrons are shown in Table 20. With the exception of the values for 1,480 rad of x-rays, each concentration is the average of at least 20 lenses. One measurement was made on four lenses given 1,480 rad of x-rays in two brief exposures of 740 rad each and consequently no standard errors are reported at this dose level.

Lens sodium in the whole mouse lens increased consistently with x-ray dose. Although potassium concentrations in the whole mouse lens do not drop much with age, it is evident that they fell drastically with increasing x-ray dose. In some cases decreases in potassium were seen

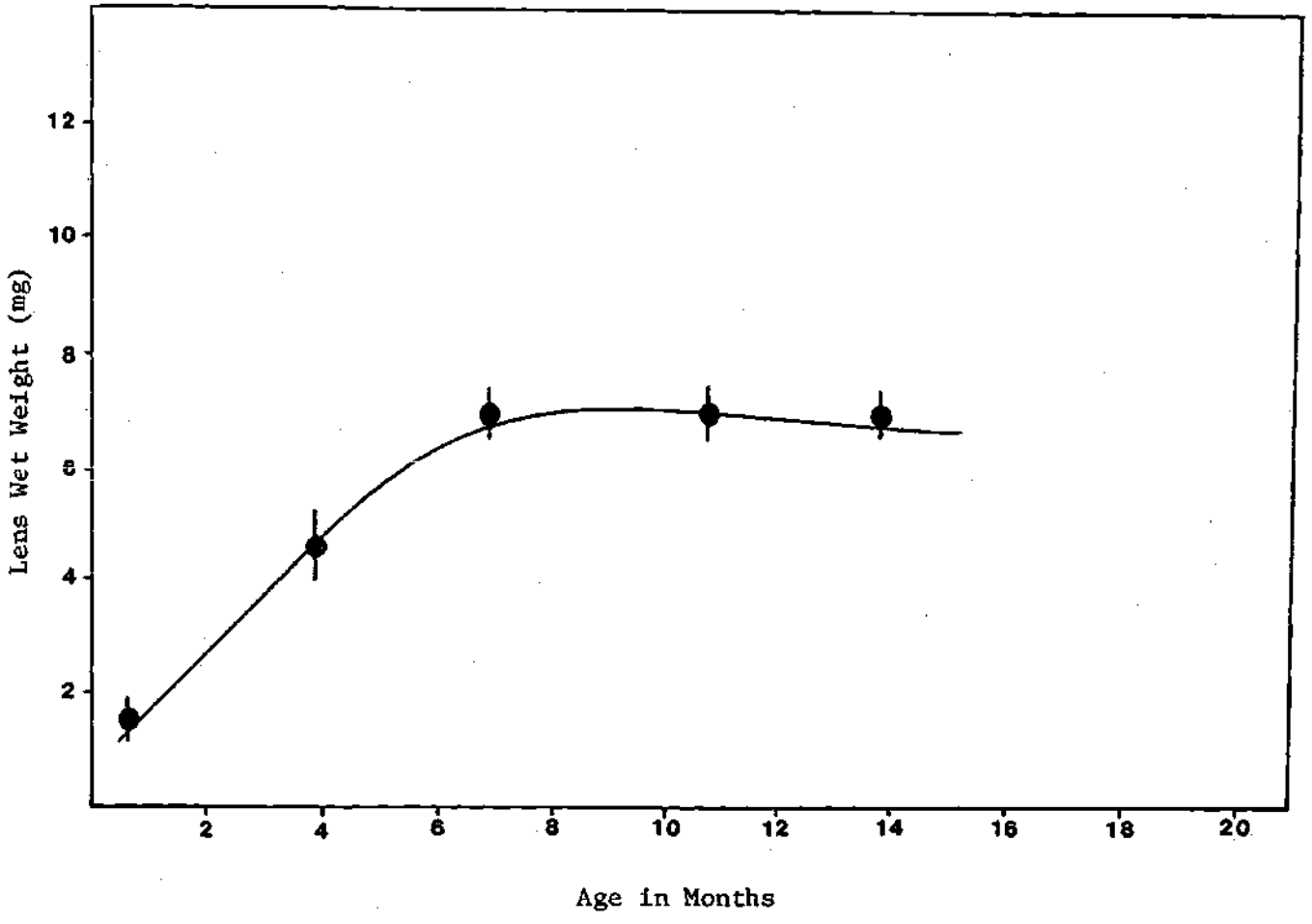


Figure 8. Variation of Mouse Lens Wet Weight with Post-Natal Age

Table 20. Variation in Concentrations† of Sodium, Potassium and Chlorine in the Adult Mouse Lens with Radiation Dose Six to Nine Months After Acute Exposure to 160 kVp X-rays, Thermal and Fast Neutrons

Dose (rad)	Radiation	Sodium	Potassium	Chlorine	K/Na
0	None	50.8 ± 6.2	181.7 ± 17.8	67.0 ± 4.6	3.6 ± 0.26
360	X-rays	69.9 ± 11.9	140.4 ± 19.1	122.4 ± 18.6	2.1 ± 0.19
740	X-rays	93.7 ± 44.2	31.5 ± 16.5	108.5 ± 16.5	0.37 ± 0.13
960	X-rays	118.5 ± 9.1	65.3 ± 15.2	97.5 ± 8.3	0.31 ± 0.21
1,480	X-rays	89.2	16.7	127.2	0.19
40	Thermal neutrons	88.5 ± 10.0	298.0 ± 16.4	100.4 ± 8.2	3.6 ± 0.57
80	Thermal neutrons	59.9 ± 7.9	208.7 ± 34.1	79.1 ± 6.7	3.6 ± 0.77
300	Fast neutrons	50.6 ± 3.6	127.8 ± 13.4	---	2.6 ± 0.36

†Concentrations are expressed as milligrams of ion ± standard error of the mean per 100 grams lens wet weight.

C57BL inbred black mice, 4-8 weeks or 20-24 weeks old at the time of exposure, were given acute doses of 160 kVp x-rays, thermal and fast neutrons in single, brief exposures. Six to nine months after exposure the animals were sacrificed and the lenses enucleated.

concurrently in lenses with increased sodium and chlorine levels. However, some lenses exhibited very low levels of potassium while possessing normal concentrations of sodium and chlorine. This implies that lenses irradiated with x-rays experience a loss of potassium prior to and irrespective of the influx of sodium and chlorine. This behavior could be linked to either a direct or an indirect inactivation of the cell membrane potassium "pump" by the x-rays. Without the active maintenance of the observed potassium levels inside cells by such pumps, the cells would quickly lose their potassium due to the diffusion gradient which exists between the intracellular and the extracellular fluids.

The potassium concentration in some of the lenses of mice exposed to an acute dose of 1,480 rad of x-rays had fallen so precipitously five months post-exposure that it was below the limits of detectability by neutron activation analysis (about 10 mg K/100 g lens wet weight or 100 ppm in this experiment).

Figure 9 is a scatter plot of the potassium concentration as a function of sodium concentration in control and irradiated mouse lenses. Each point represents a single measurement made on several lenses grouped together. It can be seen that the levels of potassium in control lenses (181.7 ± 17.8) existed within relatively narrow limits of sodium concentration, i.e., between about 27 and 90 mg Na/100 g lens wet weight. When the sodium concentration in the whole lens increased about 100 mg Na/100 g lens wet weight, however, potassium levels fell significantly and remained below normal. The failure to detect any changes in ion concentrations prior to about five months after exposure indicates that the transi-

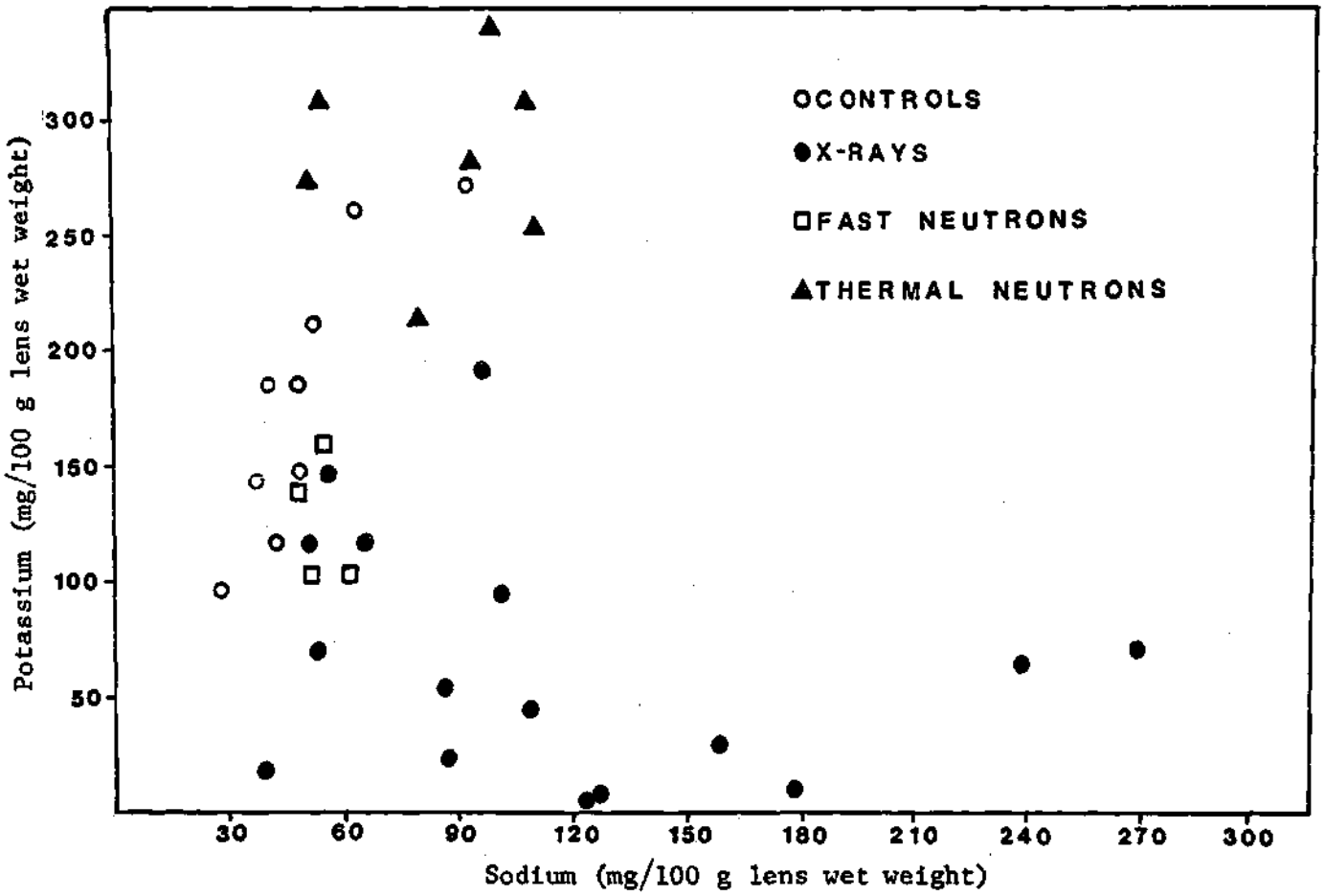


Figure 9. Potassium Concentration as a Function of Sodium Concentration in Control and Irradiated Mouse Lenses

tion from relatively normal ion levels to the severely disrupted state is delayed for some time after irradiation. Once such changes began however, they occurred rapidly.

A high positive correlation was observed between chlorine and sodium concentrations in both control and irradiated mouse lenses (see Figure 10). It is not surprising that chlorine would move chemically with sodium into lenses which have lost their ability to actively extrude sodium.

When the potassium to sodium ratio in the whole mouse lens is graphed as a function of radiation dose, however, an interesting result is obtained. The potassium to sodium ratio in the mouse lens as a function of exposure to 160 kVp x-rays, thermal or fast neutrons is shown in Figure 11. The K/Na ratio decreased from its normal value of 3.6 in control lenses to 0.19 in lenses exposed to an acute dose of 1,480 rad of x-rays. These changes occurred five to nine months after radiation exposure. A number of interpretations of this graph are possible from cell physiology considerations. Clearly some equilibrium value is reached above about 700 rad. The K/Na ratio in the whole mouse lens above 700 rad appears to approach that which exists in the aqueous humor of the eye and in blood plasma. Kuck (1970) gives a value of 0.04 for the K/Na ratio in the aqueous humor of the rabbit eye. This is also the ratio of potassium to sodium in rabbit blood plasma. If the ion transport mechanisms of the lens fail completely, passive diffusion between the lens and the aqueous humor would play a role in determining the ultimate levels of sodium and potassium within the lens.

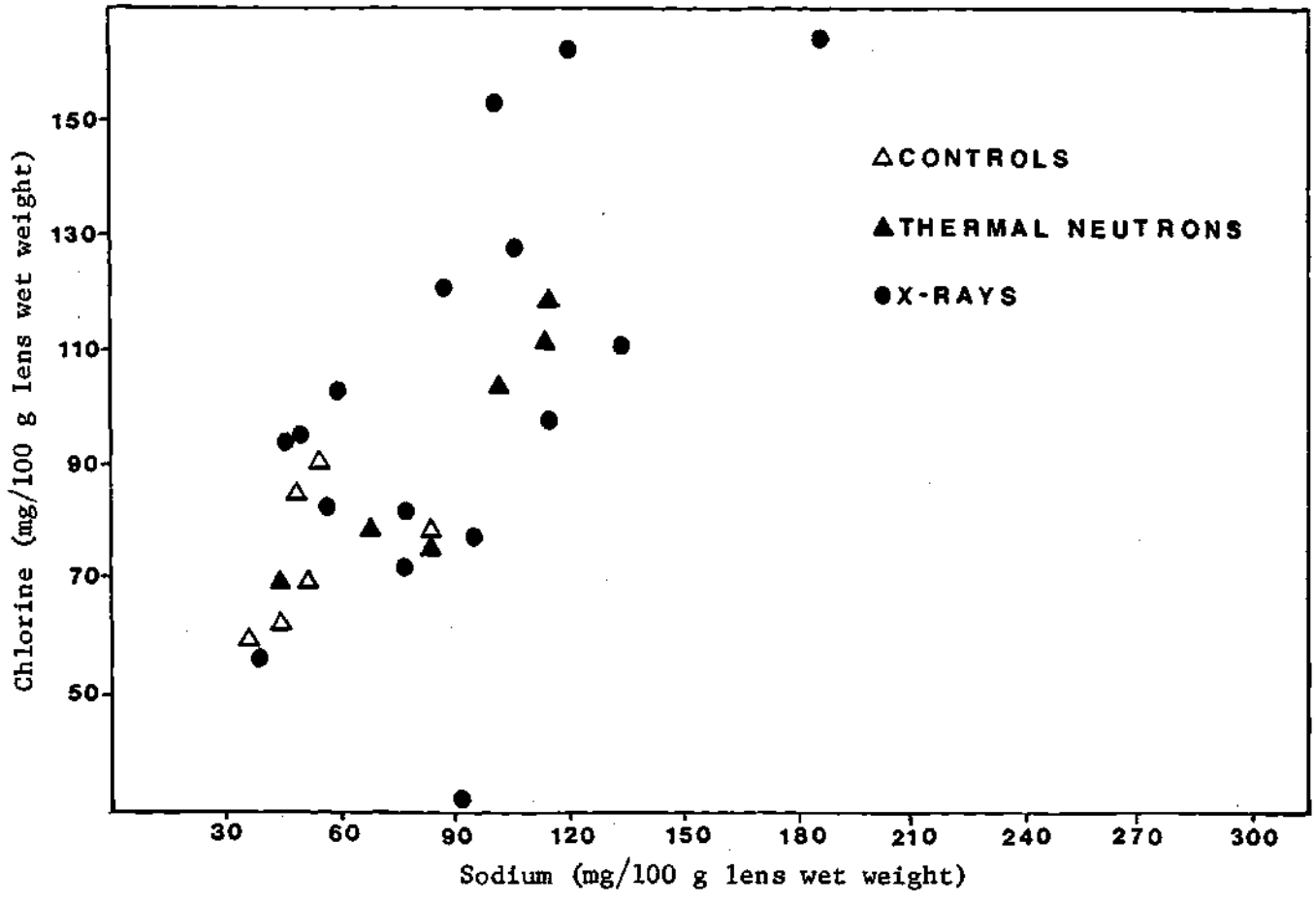


Figure 10. Chlorine Concentration as a Function of Sodium Concentration in Control and Irradiated Mouse Lenses

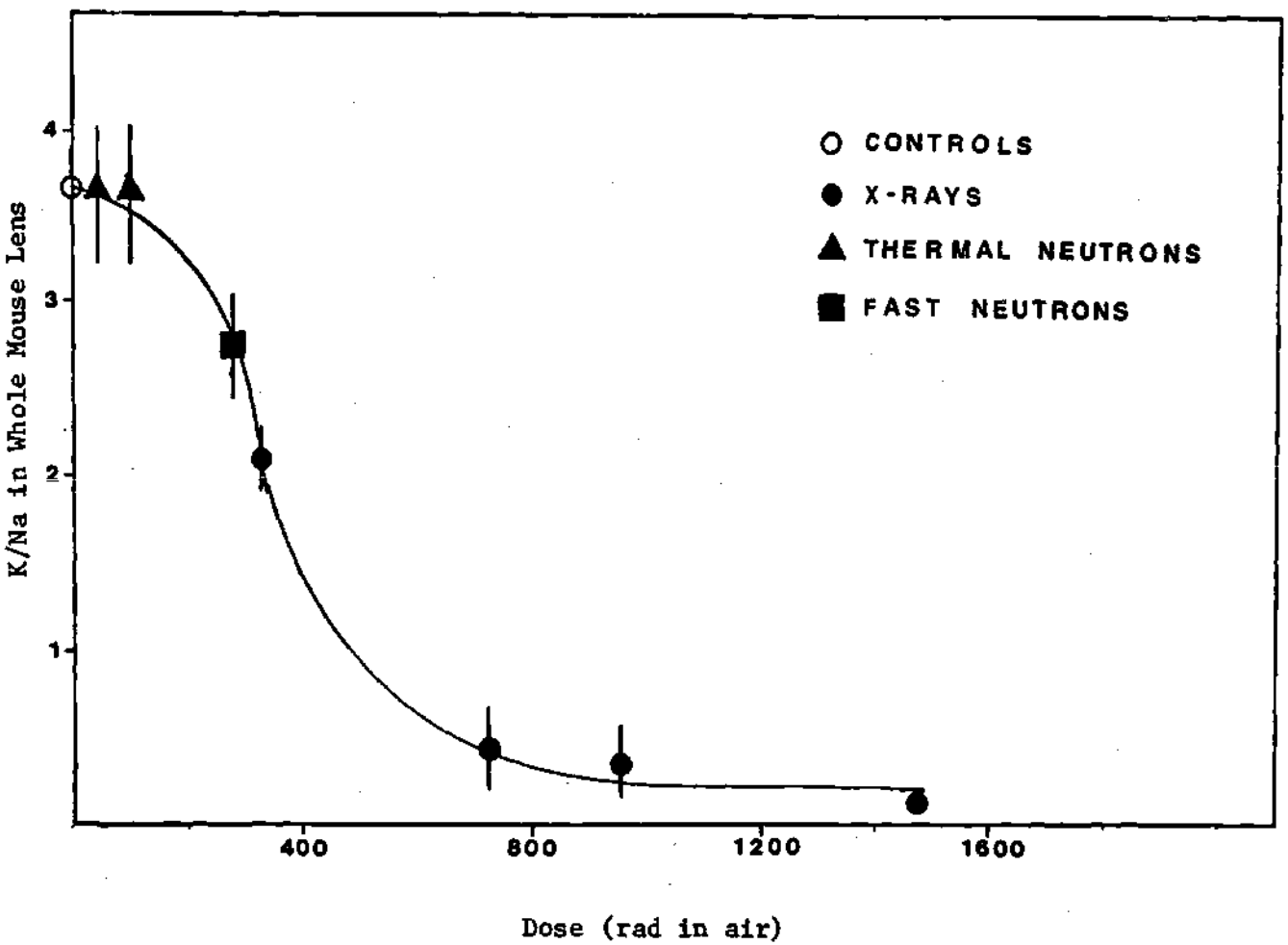


Figure 11. Potassium to Sodium Ratio in the Whole Mouse Lens as a Function of Exposure to 160 kVp X-Rays, Thermal, and Fast Neutrons

At low radiation doses (below 300 rad) it is not possible to completely rule out a nonlinear (threshold) response or, for that matter, even a biphasic response analogous to the biphasic action potential across nerve fiber membranes. The K/Na ratio has undoubtedly dropped precipitously to about 2.1 at 360 rad of x-rays.

The above findings are highly significant in light of the ionic model postulated by Duncan and Croghan (1969). These investigators described the ionic composition of the frog lens as follows:

	Lens Intracellular (mM/kg water)	Lens Extracellular (mM/liter water)
Na	18	120
K	112	4

Based on the lens membrane flux equations the lens would swell were it not for two "pumps" which are postulated--one to extrude sodium which passively enters the lens and another to pump in potassium so that one Na ion is actively extruded for every K ion pumped in. The inward K "pump" maintains a higher internal K concentration in the cell than is possible in the equilibrium state predicted by the Nernst equation. These pumps are linked in a 1:1 ratio, at least in the frog lens. The high K/Na ratio found in cells suggests that the regulating pumps are acting in such a way as to maintain the fixed negative charge concentration. These researchers suggest that cataract has two primary causes: (1) an increase in the passive sodium permeability of the lens membranes or (2) a decrease in the "pump" capacity of the lens fibers and the epithelial

cells.

Trace Elements in the Mouse Lens

A number of mouse lenses were analyzed for many of the trace elements which occur in tissue. The concentrations of chromium, antimony, scandium, cobalt, rubidium, selenium, gold and zinc in the normal adult (12 month old) mouse lens are given in Table 21. Where possible these concentrations are compared with those reported in the literature for various species. It is evident that the levels of the above elements in the mouse lens can deviate considerably from those in the other mammals which have been investigated.

Review of Accuracy and Reliability of Results

To evaluate the reliability of the observed variation of the K/Na ratio in the whole mouse and gerbil lens with radiation dose, the t-test was used to determine the significance of the difference between sample means when the sample size is small and the true population mean and variance are unknown. The K/Na ratio of 2.1 ± 0.19 in whole mouse lenses exposed to an acute dose of 360 rad of 160 kVp x-rays was found to be less than the control value of 3.6 ± 0.26, at the 98% confidence level. The values of the K/Na ratio of 0.37 ± 0.13 and 0.31 ± 0.21 for lenses exposed to acute doses of 740 and 960 rad, respectively, were found to differ from the control value at better than the 99.9% confidence level. The K/Na ratios in mouse lenses exposed to 40 and 80 rad of thermal neutrons did not significantly differ from those in control lenses.

The values of sodium and chlorine in whole gerbil lenses which had been exposed to an acute dose of 1,000 R of 160 kVp x-rays were higher

Table 21. Trace Element Content of the Normal, Adult Mouse Lens Compared with Values from the Literature for Other Animals

Nuclide	Gamma-Ray Energy (keV)	Animal	Concentration (ppm)
Cr-51	320	Mouse	0.145
Sb-122	564	Mouse	0.116
Sc-46	889	Mouse	0.220
Co-60	1,173	Mouse	0.046
		Cow	0.050 (1)
Rb-86	1,077	Mouse	0.884
Se-75	265	Mouse	1.58
		Human	0.41 (1)
		Calf	0.14 (1)
Au-198	411	Mouse	0.008
Zn-65	1,116	Mouse	34.3
		Cow	22.8 (1)
		Human	17-48 (1)

(1) Bottger et al. (1968).

than those in control lenses, at the 98% confidence level. The potassium concentration in irradiated gerbil lenses did not significantly differ from that in control lenses.

Since mouse and gerbil lenses were activated and counted in groups of three or more, it was not possible to determine the variation in results from the two lenses of the same animal. The small weight of these lenses (seven mg) made analysis of single lens samples impractical.

CHAPTER VIII

CONCLUSIONS AND RECOMMENDATIONS

The following conclusions regarding the role of the inorganic ions and the trace elements in ionizing radiation cataractogenesis in the mammalian lens seem justified on the basis of this investigation: (1) drastic and highly significant increases in sodium and chlorine and decreases in potassium occur in the mouse lens which has been exposed to high doses of x-rays and is undergoing pre-cataractous changes in chemistry and metabolism; (2) such changes in ion concentrations can be detected and measured prior to the clinical appearance of radiation cataract as detected by the slit-lamp biomicroscope; (3) similar but much less severe trends in ion levels occur in the normal mouse lens which is growing older; (4) there is evidence that mouse lenses irradiated with x-rays experience a loss of potassium prior to a net influx of sodium and chlorine. No lenses were observed to have normal potassium levels concurrent with abnormally high levels of sodium and chlorine; (5) both lenses which have clinically detectable, incipient opacities and lenses which have exhibited only pre-cataractous changes such as edema and swollen lens nuclei have ion levels which are significantly different from normal lenses; (6) apparently the important changes which occur in ion concentrations in lenses which will ultimately develop cataracts occur relatively rapidly and prior to the clinical appearance of opacities. As the opacities progress further changes in ion concentrations may occur. This may help to warn of impend-

ing cataract formation; (7) the relationship between changes in the K/Na ratio in the lens and cataract formation is still unclear and may represent parallel events, with the K/Na ratio perhaps giving an early warning of impending cataract formation. Changes in the K/Na ratio with dose may represent damage to the lens epithelium or fiber membranes and may not be directly related to cataract formation, i.e., cataracts are formed independently after exposure to high enough doses, although the K/Na ratio may serve as an indication of such an exposure.

It is recommended that further investigations be conducted into the role of the important inorganic ions in the lens undergoing cataractous changes. Special consideration should be given to the response of the K/Na ratio in the whole lens at radiation doses less than 300 rad. In particular, is there a threshold or biphasic response at low dose levels for acute exposure? What is the relation between senile and radiation induced cataracts? The behavior of the inorganic ions such as sodium and potassium should be investigated in lenses which have received x-ray exposure but were treated with cysteine prior to radiation exposure.

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VITA

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